GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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GENETICS

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VARIATION

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CORRIGENDA

Page 62, line 16, for "83" read "82."

Page 64, line 24, for "27" read "37."

Page 70, line 32, for "23.52" read "23.53."

Page 73, line 12, for "sa" read "as", line 12, for "inbotany" read "in botany."

Page 131, for "Plate 1" read "Plate 2."

Page 137, for "Plate 2" read "Plate 1."

Page 154, line 24, for " 'chromosome exchange' " read "interchromosomal crossing over or 'chromosome exchange'."

Page 156, last line, for " 'interchromosomal crossing over' " read "interchromosomal crossing over or 'chromosome exchange.' "

Page 160, last line, for " 'chromosome exchange' " read "interchromosomal crossing over or " 'chromosome exchange.' "

Page 216, line 5, after "old-gold," for the comma substitute a colon.
line 7, after "shown" use a comma instead of a period.
line, 8, for "The" read "the," and for "Ridgeway's" read "Ridgway's."
lines 9 and 11, for "strontiun" read "strontian."

Page 221, line 6 from bottom, for "five" read "six."

Page 225, first line of second paragraph, for "five" read "seven."

Page 230, line 11 from bottom, for "(1923)" read "(1922)."

Page 242, line 10, for "Percentage crossing over equals 110" read "Percentage crossing over equals 100."

Page 286, line 7, for "purple" read "not-purple."

Page 289, line 1, for "Median" read "Middle."
line 3, for "median" read "extra middle."
line 4, for "median" read "middle."

Page 290, lines 7–9, for "usually vertical—*vice versa*" read "dorso-ventral."

Page 331, second line of summary, for "with other" read "with certain other."

Page 317, line 4 from bottom, for "is always 7 so far as known" read "is 7."

Page 330, first line of last paragraph, for "proportion" read "proportions of,' and for "generation" read "generations."

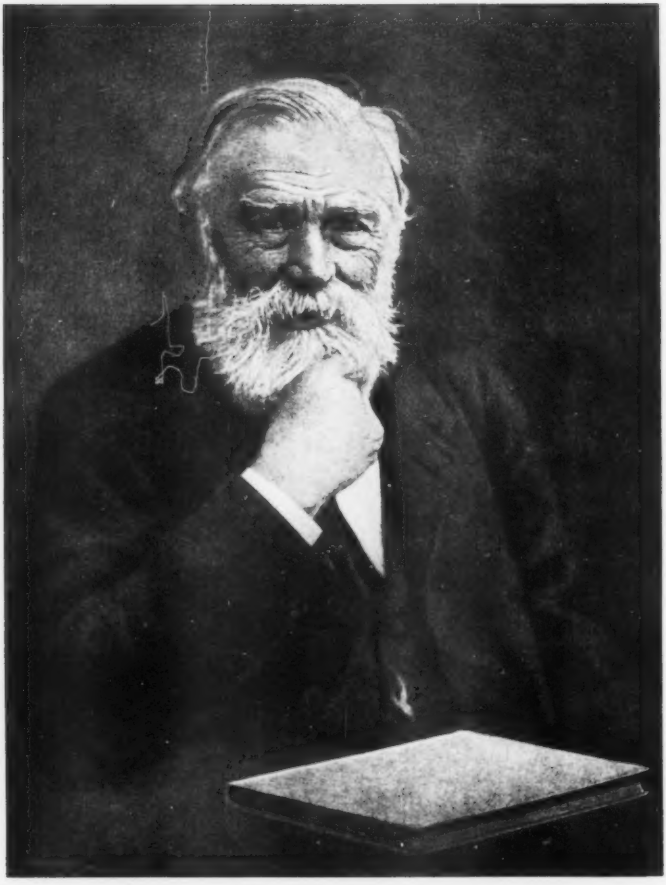
Page 356, line 6, omit comma after blue.

Page 358, line 10, "only" should be followed by a comma.

Page 368, line 3 from bottom, for "four genotypes, namely, BBLLee, BBLlee, BBllEE, and BBllEe," read "two genotypes, namely—BBLLee and BBLlee."

Page 423, for "Received March 1, 1925" read "Received March 1, 1926."

Page 502, Bibliography incomplete.



Hochachtungsvoll

Dr. W. O. Focke

WILHELM OLBERS FOCKE

(FRONTISPIECE)

Medizinalrat Doctor WILHELM OLBERS FOCKE was born April 5, 1834, in Bremen, Germany, where he died September 29, 1922. All of his life was spent in or near Bremen, except his school years, 1853 to 1858, in Bonn, Würzburg, Vienna and Berlin. He was a practicing physician in Oberneuland 1859–1861 and then in Bremen; Director of the BREMEN HOSPITAL, 1864–1867; Police Physician, 1871–1886; Prison Physician at Oslebshausen, 1874–1901; Member of the BOARD OF HEALTH of Bremen, 1886–1904; Business Manager and Consulting Physician to the BOARD OF HEALTH, 1901–1904; Directing Physician and Vice-President of ST. JURGEN'S ASYLUM, 1896–1897.

Although pure science was only his avocation, he accomplished an amazing amount of work, not only in botany and genetics, but he was also author of many papers on the geology, topography and climatology of Lower Saxony, and of biographical sketches of many of the distinguished scientific men of Bremen.

In 1864 he was one of the founders of the SCIENTIFIC SOCIETY OF BREMEN, and was most active in his participation in its activities. He edited its proceedings until 1895 and for many years gave numerous brilliant and finished lectures before its sessions. Shortly after he retired from the editorship of the Proceedings he was elected an Honorary Member of the Society.

He accepted the views of DARWIN before these had gained general recognition and many of his earlier papers, published mostly in "Kosmos" and the Proceedings of the AUSTRIAN BOTANICAL SOCIETY, discussed species formation and other matters relating to organic evolution.

While FOCKE is best known to geneticists for his critical compilation, "Die Pflanzen-Mischlinge," published in 1881, his greatest contribution was undoubtedly the application of genetical methods to taxonomic problems in such difficult polymorphic genera as Rosa and Rubus. "Die Pflanzen-Mischlinge" has the honor of having been not only for several decades the most important source-book and starting-point for researches in hybridization, but, according to the account of DE VRIES, this book formed an important connecting-link between MENDEL'S classic paper on the garden pea and the "rediscovery" of Mendelian heredity in 1900, for DE VRIES found the reference to MENDEL'S paper in L. H. BAILEY'S "Plant Breeding" (published in 1895), where it had been cited from FOCKE'S "Pflanzen-Mischlinge."

In this work FOCKE referred to MENDEL'S papers as "particularly instructive" but gave no indication as to the nature of their instructiveness, and, strangely enough, praised GODRON'S studies as of the greatest importance in showing that true-breeding forms can result from hybridization, a fact best established by the wonderfully clear analysis given by MENDEL. To the end of his life FOCKE remained indifferent to the problems of analysis of hybrids on the basis of single characteristics. His interest was centered more on the significance of hybridization as a species-forming process and in relation to taxonomy.

He began his important studies on the taxonomy of Rubus early and published his "Synopsis Ruborum Germaniae" in 1877, and did not rest his extensive labors with this important and difficult genus until the completion of his "Species Ruborum Monographiae Generis Rubi Prodromus," published in three parts in Bibliotheca Botanica 1910–1914. The great value of this

work rests on FOCKE's extensive utilization of progeny tests for constancy of differentiating characters, and numerous hybridizations to determine relationships. He was scientifically active almost to the end of his life, and as late as 1920 he published a brief paper on "Old disappearing and newly arising plant species."

The photograph here reproduced was taken in 1907 on the occasion of Doctor FOCKE's Doctorial Jubilee. We are indebted to Doctor H. DUNCKER, of the UNIVERSITY OF BREMEN, and, through his mediation, to the relatives of Doctor FOCKE for the loan of such portraits as are available, thus making possible a choice of the one which seemed best adapted to our use. Doctor G. TISCHLER of the UNIVERSITY OF KIEL kindly loaned a prized letter from which the signature has been reproduced.

THE VARIABILITY OF CROSSING OVER IN MAIZE*

L. J. STADLER
College of Agriculture, University of Missouri, Columbia, Missouri

Received May 9, 1925

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INTRODUCTION

This study is concerned chiefly with the extent and nature of the variability of crossing over in a family (full sibship) of *Zea mays*. It is part of a general study of variation in crossing over in maize, in which it is proposed to parallel the variousstu dies of this problem in Drosophila, as far as the nature of the material will permit, and also to investigate certain phases of the problem for which the plant is well suited and the fly is not.

Although the excellent conditions for the experimental study of linkage in Drosophila cannot be duplicated in maize, with its much longer reproductive cycle and much smaller known linkage groups, the plant offers quite favorable material for work of this kind in one linkage group of three endosperm characters. As far as the inheritance of endosperm characters is concerned, the single grain is the individual, and each well-developed ear comprises a progeny of several hundred. Thus it is possible, when using these characters, to handle the large numbers necessary in a statistical study. EAST (1913) has shown that "double fertilization" regularly occurs in maize, and that endosperm characters, therefore, may safely be used to indicate the genetic constitution of the zygote.

The three linked genes used in this study are *C c*, which in the presence of certain other genes affects aleurone color; S_h s_h, which affects the degree of shrinkage of the endosperm at maturity; and W_x w_x which affects the

* The GALTON AND MENDEL MEMORIAL FUND has contributed toward the cost of setting the tables accompanying this article.

chemical composition of the endosperm. Their linkage relations, which have been studied extensively by KEMPTON (1919), HUTCHISON (1922), and others, are indicated in figure 1.

FIGURE 1.—Linkage relations of the factors C, S_h and W_x. (After HUTCHISON 1922).

The crossover percentages in these two sections of the chromosome are not ideal for the purpose, one being lower and the other somewhat higher than might be desired. The low crossover percentage is undesirable because the probable error computed by the usual formula,

$$E = .6745\sqrt{\frac{pq}{n}}$$

is unreliable when p is very small as compared with q, unless n is very large. The significance of differences in crossing over in this region is, therefore, somewhat doubtful when estimated by the use of this formula. The higher crossover percentage of the S_h-W_x region is undesirable because of the danger that undetected double crossovers may occur within the region. Since no locus between S_h and W_x is known at present, we cannot determine how frequently double crossing over may occur within this region. But double crossovers involving simultaneously the C-S_h and S_h-W_x regions are extremely rare, as HUTCHISON (1922) has shown and as the data of this paper confirm. It therefore seems probable that double crossing over within the S_h-W_x region is negligible as a source of error, although of course it is possible that double crossing over may occur freely in part of the C-W_x region while partly or wholly inhibited in another part.

The chief advantages of the material for the study of linkage problems are:

(1) Crossing over occurs freely in the development of both "male" and "female" gametes, and, since the plant is monoecious, both male and female gametes are produced by the same individual.

(2) A very large progeny may be produced from a single individual. The grains produced by a single heterozygous plant (which, backcrossed, indicate the crossover percentage among the female gametes) number one to two thousand in well-developed plants. The number of grains of multiple-recessive plants which may be pollinated from a single heterozygous plant (and which then indicate the crossover percentage among male gametes) is almost unlimited. By pollinating several ears it is possible to

produce a progeny of 5000 or more in each day's pollination throughout the pollen-shedding period, which, in well-developed plants, often lasts 20 days or more.

(3) Since pollination is easily effected and the sorting of the progenies may be done at one's convenience, it is practicable to determine the variability of crossing over in a large number of individuals of a single family grown simultaneously under uniform conditions.

(4) Tillers may be transplanted in early stages, and these under favorable conditions produce both seed and pollen. Separated parts of the same individual therefore may be exposed to different treatments to determine the effects of the treatments on crossing over.

(5) Samples of the young anthers can be taken at the time of the reduction divisions or even earlier, with no injury to the breeding powers of the plant. The stage in the development of the germ cells and the cytological effects of treatments may therefore be determined in the plants which are later to be used in breeding.

The series of studies of which this paper is a partial report was made possible through the generosity of Professor R. A. EMERSON, who furnished me the foundation stock of this favorable material. I am indebted also to Professor EMERSON and to Professor E. M. EAST for criticism of the manuscript.

PREVIOUS STUDIES OF VARIATION IN CROSSING OVER

Crossing over has been found highly variable in Drosophila. GOWEN (1919), in an extensive biometrical study of crossing over in the third chromosome, found coefficients of variability ranging from 18 to 59 for the percentage of single crossovers in various regions, and from 67 to 110 for the percentage of double crossovers. In general the crossover percentages for the shorter regions were more variable than those for the longer regions. GOWEN concluded:

> "Crossing over is one of the most variable phenomena known, indicating that the mechanism behind crossing over is not as precise as that found in most physiological studies."

DETLEFSEN also found crossing over in Drosophila "an enormously variable phenomenon" as he remarks in reporting his experiments on selection for high and low crossing over in the sex chromosome (DETLEFSEN and CLEMENTE 1923).

The relation of specific environmental and genetic factors to crossing over in Drosophila has been studied by a number of investigators. A decrease in crossing over with age, limited to certain chromosome regions, was found by BRIDGES (1915). A striking increase in crossing over under

extreme temperatures, either high or low, was found by PLOUGH (1917, 1921), this effect being limited to the "susceptible" regions in which crossing over was affected by age. In PLOUGH'S experiments no significant difference in crossing over was found to result from varying amounts of moisture in the food, starvation, increased fermentation of food, or the presence of ferric chloride. MAVOR (1923) and MAVOR and SVENSON (1924) found X rays effective in modifying the crossover percentage in both the second chromosome (susceptible to age and temperature effects) and the first chromosome (not susceptible to age and temperature effects). The same X-ray treatment increased the crossover percentage in the second chromosome and decreased it in the first. PLOUGH (1924), in a preliminary trial of the effect of radium radiations, found a general tendency toward increased crossing over, especially after severe radiation.

Linkage variations due to germinal causes have been investigated especially by STURTEVANT and DETLEFSEN. STURTEVANT (1913, 1915, 1917, 1919) has reported several genes modifying the crossover percentage. The first gene of this kind reported, C_{III}, greatly decreases crossing over in the right-hand end of the third chromosome in flies which are heterozygous for it, though in flies homozygous for it crossing over in the same region is at least as high as normal. Similar results are given by C_{II_r} and C_{III_l}, which affect crossing over in the right-hand end and left-hand end, respectively, of the second chromosome, though the homozygous effect of C_{III_l} has not been determined. Two other crossover modifiers in the third chromosome have also been reported, C_{III_p} by PAYNE (1918) and C_{III_M} by BRIDGES and MORGAN (1923). These genes also reduce crossing over when heterozygous. Recently, STURTEVANT (1921) has suggested the possibility that the reduction in crossover percentage ascribed to the action of C_{III} and C_{II_r} may be due rather to an inverted chromosome section, which might prevent synapsis when present in only one chromosome of a pair but not when present in both.

DETLEFSEN (1919), DETLEFSEN and ROBERTS (1920, 1921), and DETLEFSEN and CLEMENTE (1924) have reported experiments in which the crossover percentage in the sex chromosome was greatly reduced by selection. Selection for increased crossing over was unsuccessful, a result attributed to increased double crossing over. A cross of "normal" stock with one of the low-crossover stocks produced by selection, gave results considered indicative of multiple-factor inheritance.

GOWEN and GOWEN (1922) have reported a third-chromosome gene which apparently inhibits crossing over completely in the female. They suggest that the results of DETLEFSEN'S experiments may be accounted for by the action of one or more genes of this type.

NABOURS (1919) reported that crossing over in Apotettix was apparently more frequent in the female than in the male. CASTLE (1919) also found that crossing over was more frequent in the female than in the male in rats. At the time, CASTLE doubted that this difference would be shown under uniform conditions, but in a later and more extensive study, CASTLE and WACHTER (1924) found the higher crossover percentage in females to be consistent and to occur in mice as well as rats. No evidence of any relation of crossing over to age or seasonal conditions was found. Variability in crossing over in different male rats was found to be no greater than could be accounted for as the result of random sampling, with the possible exception of one individual.

Studies of variation in crossing over in plants have thus far been limited to comparisons of the crossover percentages in male and female gametes. GOWEN (1919) pointed out that in the data given by ALTENBURG (1916) for linkage in Primula, the percentage of crossovers in the male gametes tested was significantly higher than that in the female gametes. BATESON (1921) stated that extensive data on linkage of magenta color and short style in Primula show closer linkage in the eggs than in the pollen, but that the linkage of green stigma and reddish stem is closer in the pollen than in the eggs. EYSTER (1921) in a study of the linkage relations of sugary endosperm and tunicate ear in maize, found that the crossover percentage was 7.9 ± 2.0 higher among male than among female gametes. He also compared the C-W_x crossover determinations of BREGGER (1918) in a similar way and found that in these trials the crossover percentage was 2.46 ± 1.11 percent higher in the male gametes.

In all of these trials one group of plants used as male parents was compared with another group used as female parents. EMERSON and HUTCHISON (1921), pointing out that the results of such experiments may be due to environmental or genetic differences other than sex, submit data on crossing over in the production of male and female gametes in the same plants. Using the linkage of plant color and liguleless leaf in maize they found no significant difference in crossing over in the production of male and female gametes. In the linkage of colored aleurone and shrunken endosperm the percentage of crossovers was found to be slightly, but significantly, higher among the female gametes. They point out that this may be due to disturbing conditions such as temperature effects rather than to inherent difference in the mechanism of crossing over. EYSTER (1922) has also supplemented his earlier study with a comparison of the crossover percentage in the S_u-T_u region in male and female gametes of the same plants, in which he found no significant difference between the sexes.

MATERIAL AND PROCEDURE

The family of plants used in this study (pedigree number 4003) was grown from seeds of a single ear. This ear was produced by pollinating a plant (1598-14) homozygous for colored aleurone, non-shrunken, starchy endosperm (*C C* S_h S_h W_z W_z) by a plant (1804-1) homozygous for colorless aleurone, shrunken, waxy endosperm (*c c* s_h s_h w_z w_z). Both parents were also homozygous for the genes *AA*, *RR*, and *ii*, the factors complementary to *C* in the production of aleurone color.

The two parental strains have been in genetic cultures for some years past. Family 1804, to which the male parent belonged, was grown from seed obtained from Doctor EMERSON in 1923. Family 1598, which included the female parent, was derived from a stock obtained in 1921 from Doctor E. W. LINDSTROM, who had grown it for several seasons. Through the kindness of Doctor EMERSON and Doctor LINDSTROM I have secured the pedigrees of these strains, which are shown in figure 2.

In order to favor maximum development of individual plants, the crop was planted one grain to a hill, 42 inches apart each way. The soil, which

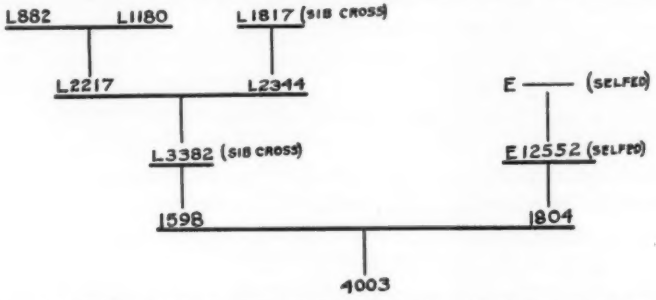

FIGURE 2.—Pedigree of family 4003. Pedigree numbers preceded by E refer to cultures of EMERSON; those preceded by L to those of LINDSTROM.

was naturally fairly fertile, was further improved by the application of a 4-12-3 fertilizer at the rate of 600 pounds per acre shortly before planting. Two rows of 50 hills each were planted to the heterozygous stock. About 75 of the 100 plants reached the flowering stage and all of these were used without selection.

The crossover determinations were made by backcrossing with a multiple-recessive stock. The crossover percentage in male gametes was determined by pollinating at least one ear of the recessive stock from each tassel which shed pollen; in female gametes by pollinating two ears of the main stalk and the first tiller ear of all of the heterozygous plants

which produced these ears. Of the heterozygous plants 58 produced 2 ears on the main stalk, and 31 produced a tiller ear. The multiple-recessive stock used was of low vigor and produced rather small ears. Some of these plants were so injured by chinch-bugs that they produced practically no grain. Consequently, the progenies representing the crossover series in the male gametes are smaller than those representing the female series, and for a few of the plants no determination of crossover frequency in male gametes could be made.

Pollen of the heterozygous plants was used regularly on the third day following the beginning of pollen shedding, to avoid error from any possible tendency towards change in crossing over with age of plant or inflorescence. These pollinations were made daily between 10 a.m. and noon. The ears of heterozygous plants were pollinated when the silks seemed well-enough developed to insure good filling of the ear.

In the sorting of the backcross progenies, each grain was individually tested for composition of endosperm (starchy or waxy) by a modification of the method suggested by WEATHERWAX (1923). Before shelling each ear a spot on each kernel was scraped to expose the endosperm, an operation quickly accomplished by the use of a small emery wheel. Before shelling and sorting the grains of each row, they were tested for endosperm composition by applying a touch of iodine solution to the scraped spot of each grain. The separations for aleurone color and shrinkage of the endosperm were clear and unmistakable except in a few ears of the heterozygous plants in which the shrinkage of the endosperm was very slight. These ears were separated only for *C c* and W_z w_z, S_h s_h being disregarded.

VARIABILITY OF CROSSOVER PERCENTAGE IN MALE GAMETES

The crossover percentage in male gametes, as shown by backcrossing to the multiple-recessive stock, was determined for 59 plants. The classified progenies of these plants are shown in table 14 (appendix). The extreme range in crossover percentage and the variability of crossing over in the *C*-S_h, S_h-W_z, and *C*-W_z[1] sections are shown in table 1 (page 8).

The variability of crossover percentages, as measured by the coefficient of variability, is decidedly higher in the shorter region than in the longer, as was found by GOWEN in Drosophila. In both the *C*-S_h and the S_h-W_z regions the variability is considerably less than that found by GOWEN in

[1] The crossover percentage for the *C*-W_z region is determined by summation from those of the component regions (deducting twice the percentage of double crossovers). It is given to permit direct comparison with the plants in which shrunken endosperm could not be distinguished.

TABLE 1

Variability of crossover percentage in male gametes.

REGION	HIGHEST PERCENTAGE	LOWEST PERCENTAGE	MEAN PERCENTAGE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
C-S_h	6.3	0.4	3.271 ± 0.101	1.148 ± 0.071	35.10 ± 2.43
S_h-W_x	30.3	13.8	22.115 ± 0.263	2.992 ± 0.186	13.53 ± 0.85
C-W_x	33.1	14.2	25.117 ± 0.265	3.021 ± 0.188	12.03 ± 0.76

Drosophila in regions of similar length. For example, in the three sections of about 20 units length reported for Drosophila, the coefficients of variability were 31.0, 30.3, and 24.3, as compared with 13.5 in the S_h-W_x region of maize, and in the one region of about three units length reported for Drosophila the coefficient was 59.0, as compared with 35.1 for the C-S_h region in maize.

This lower variability of crossover percentages in the maize data does not necessarily mean that crossing over is a less variable process in maize than in the fruit fly, though of course this is a possibility. The two trials were under very different conditions and are not directly comparable. Two conditions in particular tend to make the variability lower in the maize data: (1) the plants compared are of the same parentage and are grown simultaneously under uniform conditions; and (2) the backcross progenies used in determining the crossover percentages are larger, so that the part of the variability which results from fluctuations of sampling is reduced.

The latter point may require some further explanation. Suppose we are dealing with a family of plants in which crossing over is perfectly uniform; that is, each plant *produces* exactly the same percentage of crossover gametes. Now in a study of the variability of crossing over in this family, we determine the crossover percentage in each plant from a backcross progeny of 400. The crossover percentages thus determined will not be uniform; they will fluctuate about the true value, with deviations dependent upon the size of the samples. For example, if the proportion of crossover gametes actually produced by each plant were 20 percent, the crossover percentage determined for each plant, from a backcross progeny of 400, would have a probable error of 1.33 crossover units. If crossover percentages were thus determined for 100 plants, about 50 of them would be below 18.67 or above 21.33 (20.00 ± 1.33) and about 4 of them would be below 16 or above 24 ($20.00 \pm 3 \times 1.33$). Thus, there would be considerable variation in the crossover percentages determined, but it would be variation of sampling, not variation of crossing over. The series of 100

crossover values would have a mean of about 20 crossover units, a standard deviation of about 2 crossover units, and therefore a coefficient of variability of about 10 percent. If backcross progenies of only 100 instead of 400 had been used, the variability due to error of sampling, determined in the same way, would have been about 20 percent instead of 10 percent. If a chromosome region 10 units long, instead of 20, had been used, with backcross progenies of 100, the population of plants, though actually perfectly constant in crossing over, would have given a coefficient of variability of about 30 percent. Thus, when crossover frequency is measured by means of progenies of moderate size, one may find rather high apparent variability, though no lack of precision in the mechanism of crossing over is involved. This is true especially of the shorter chromosome sections, which explains, at least in part, the high variability found in the C-S_h section and in the short sections studied in Drosophila by GOWEN. Probably if large enough progenies were used in the measurement of crossover frequency in the short sections, their crossover percentages would be found no more variable than those of the long sections. But very large progenies would be required. In order to reduce to 10 percent the variability due purely to random sampling, progenies of more than 3000 each would be required for a section 3 units long, while progenies of 400 each would suffice for a section 30 units long.

The foregoing considerations suggest that crossing over may actually have been a very uniform process in the family of maize plants studied, and that such variations as were found may have resulted largely or wholly from error of sampling. Although the coefficients of variability determined are larger than would be expected to result from error of sampling alone, with samples of the size used, it is of interest to determine to what extent the variations may be accounted for by sampling fluctuations.

If all of the plants were actually identical in crossing over, (that is, if the variations found resulted wholly from error of sampling), only about half of the percentages of crossing over determined for individual plants should deviate from the mean percentage by as much as their probable errors, about 18 percent by as much as twice their probable errors, and so on, the expected frequencies being determined from tables of the probability integral. The actual frequency of such deviations, compared with the frequency expected if random sampling were the only cause of variability, is shown in table 2 on page 10.

It is clear that the variation between plants is wider than would be expected to result from error of sampling. In other words, the variability

in crossover percentage found, though due in part to fluctuations of sampling, is due in part also to actual differences in the frequency of crossing over in different plants.

The correlation between crossover percentage in the C-S_h and S_h-W_z regions, in the male gametes of these 59 plants, was $-.247 \pm .082$. There

TABLE 2

Variability of crossover percentage in male gametes compared with variability expected as result of error of sampling.

REGION	DEVIATION FROM MEAN, DIVIDED BY PROBABLE ERROR				
	1 or more	2 or more	3 or more	4 or more	5 or more
Expected	*29.50*	*10.46*	*2.54*	*0.41*	*0.04*
Observed:					
C-S_h	37	18	10	3	1
S_h-W_z	36	19	6	3	0
C-W_z	35	19	5	2	1

was thus a slight but possibly significant tendency for a high crossover percentage in one section to be accompanied by a relatively low crossover percentage in the adjoining section.

Variations in crossover percentage were not significantly correlated with height of plant, number of tillers, or number of ears. There was wide variation in vigor of growth among the plants of this family, plant height varying from 5 to 8½ feet, number of tillers from 0 to 4, and number of ears (including all which produced any grain whatever) from 1 to 8. But these differences had no discernable relation to the frequency of crossing over. There was a slight but possibly significant negative correlation of date of first pollen shedding and crossover percentage for the S_h-W_z region. The crossover percentage for C-S_h, which, as stated above, was negatively correlated with that for S_h-W_z, did not show any relation to date of first pollen. The correlation coefficients determined are shown in table 3.

TABLE 3

Correlation of crossover percentage in male gametes with certain plant characters.

PLANT CHARACTER	CORRELATION WITH CROSSOVER PERCENTAGE		
	C-S_h	S_h-W_z	C-W_z
Date of first pollen	$-.038 \pm .092$	$-.255 \pm .086$	$-.246 \pm .086$
Plant height	$.007 \pm .091$	$-.122 \pm .090$	$-.141 \pm .089$
Number of tillers	$.060 \pm .090$	$.134 \pm .089$	$.178 \pm .087$
Number of ears	$.022 \pm .090$	$.013 \pm .090$	$.036 \pm .090$

VARIABILITY OF CROSSOVER PERCENTAGE IN FEMALE GAMETES

A similar study was made of variation in crossover percentage in female gametes in this family. Crossover percentages in the tiller ears were found to differ significantly from those in ears of the main stalk, as will be shown presently. Since many of the plants did not produce tiller ears, it was necessary to disregard all tiller ears in determining the crossover percentages to be used in the study of variability. The crossover percentage in ears of the main stalk was determined in 70 plants. In 7 of these the shrunken character was indistinct, and was disregarded in making separations. There are available for the study of variation in crossover frequency in female gametes, therefore, crossover percentages in all three of the regions studied, in 63 plants, and in the *C* W_x region only in 7 additional plants. The classified progenies are shown in table 15 (appendix).

The variability of the crossover percentages thus determined is shown in table 4.

TABLE 4

Variability of crossover percentage in female gametes.

REGION	NUMBER OF PLANTS	HIGHEST PERCENTAGE	LOWEST PERCENTAGE	MEAN PERCENTAGE	STANDARD DEVIATION	COEFFICIENTS OF VARIABILITY
C-S_h	63	5.8	0.4	2.321±0.082	0.966±0.058	41.62±2.91
S_h-W_x	63	28.6	14.8	20.000±0.242	2.851±0.171	14.26±0.87
C-W_x	63	30.7	15.7	22.156±0.264	3.1[illegible]2±0.186	14.00±0.86
C-W_x	70	30.7	15.7	22.374±0.257	3.187±0.182	14.24±0.83

The variability is of the same order as that found in male gametes, though in all cases the coefficients of variability in the female gametes are slightly higher. This slight increase in variability might perhaps be accounted for by increase in sampling fluctuations accompanying the lower mean crossover percentages found in female gametes. On the other hand, sampling fluctuations should be reduced somewhat by the larger progenies used in determining crossover percentages in the female gametes.

The extent to which fluctuations of sampling may account for the variations observed is better shown by direct comparison of the observed deviations from the mean with the deviations expected from sampling errors alone. These are compared in table 5, which is prepared in the same way as table 2 for the male gametes.

TABLE 5

Variability of crossover percentage in female gametes compared with variability expected as result of error of sampling.

REGION	DEVIATION FROM MEAN, DIVIDED BY PROBABLE ERROR							
	1 or more	2 or more	3 or more	4 or more	5 or more	6 or more	7 or more	8 or more
Expected	*31.50*	*11.17*	*2.71*	*0.44*	*0.05*	*0.00+*	*0.00+*	*0.00+*
Observed:								
C-S_h	44	29	16	6	3	0	0	0
S_h-W_z	45	32	16	9	4	1	1	1
C-W_z	43	32	20	12	5	2	1	1

The variability is well beyond that which may be accounted for by fluctuations of sampling. Obviously, the crossover percentage in female gametes, like that in male gametes, varied among individual plants within the population. The extent of variation in crossover percentage beyond that expected from sampling fluctuations is greater in the female gametes than in the male gametes.

The fact that there were individual differences between the plants of this family is further shown by the correlation between crossover percentages in different ears of the same plant. If crossover variations resulted only from error of sampling, different ears of the same plant should be no more similar than ears of different plants, for all ears would be simply random samples from the same population. In other words, if fluctuation of sampling were the only cause of differences between plants, the crossover percentages of different ears of the same plant would be uncorrelated variables. But actually the crossover percentages in different ears of the same plant were highly correlated. The 52 plants which produced two ears of the main stalk gave the following correlations for crossover percentage in the first and second ear:

Region	r
C-S_h	$.451 \pm .078$
S_h-W_z	$.614 \pm .061$
C-W_z	$.686 \pm .048$

These clearly significant correlations show that plants within this family differed significantly in crossing over, though they do not show whether such differences were due to germinal or environmental causes.

In the 63 plants in which crossover percentages for both C-S_h and S_h-W_z were determined, the correlation of crossover percentages in the two regions was $.257 \pm .081$. This is contrary to the condition found in the

male gametes, in which the crossover percentages in these two regions were negatively correlated to about the same extent.

The relation of crossover percentage in female gametes to plant height, number of tillers, number of ears, and date of first pollen shedding is shown in table 6.

TABLE 6

Correlation of crossover percentage in female gametes with certain plant characters.

PLANT CHARACTER	CORRELATION WITH CROSSOVER PERCENTAGE		
	C-S_h	S_h-W_x	C-W_x
Date of first pollen....	.017 ± .088	.130 ± .086	.122 ± .087
Plant height.....	−.153 ± .085	−.348 ± .077	−.340 ± .077
Number of tillers.....	−.094 ± .086	−.179 ± .084	−.180 ± .084
Number of ears.....	−.193 ± .083	−.261 ± .080	−.296 ± .079

There is clearly a tendency toward negative correlation of crossover percentage and characters indicating vigor of growth, though all of the correlation coefficients are low. Plant height shows the closest correlation with crossover percentage, but number of ears and number of tillers also show some correlation, at least in the S_h-W_x and C-W_x regions. The correlation coefficients are lower for the C-S_h region, as was found also in the correlations determined above for crossover percentage in different ears of the same plant. The probable reason for this lower correlation in the C-S_h region is that the variations in this short region were more largely the result of sampling fluctuations than the variations in the longer regions. Date of first pollen was not significantly correlated with crossing over.

RELATIVE FREQUENCY OF CROSSOVERS IN MALE AND FEMALE GAMETES

The data already presented show a higher mean crossover percentage in male gametes than in female gametes. The data given for female gametes include several plants in which crossover frequency in male gametes was not determined, and those for male gametes include a few plants in which female gametes were not tested. Crossover percentages in both male and female gametes were determined in 54 plants, including 5 in which separations for S_h s_h could not be made in the ears representing the female gametes. These determinations are shown in table 16.

The low correlation between crossover percentages in the male and female gametes of the same plant is striking. The correlation coefficients are as follows:

Region	*Number of plants*	*Coefficient of correlation*
C-S_h	49	.212±.092
S_h-W_x	49	.075±.096
C-W_x	54	.122±.090

This is in agreement with the results of EMERSON and HUTCHISON (1921) on crossing over in the B-l_g and C-S_h regions in maize. These investigators found in plants extreme in crossover frequency in the gametes of one sex no marked tendency toward extreme results in the other sex. Correlation coefficients computed from the data given by them follow:

Region	*Number of plants*	*Coefficient of correlation*
B-l_g	19	.358±.135
C-S_h	20	.185±.146

The crossover percentages in male and female gametes of the same plants, computed from the totalled progenies of table 16, are shown in table 7.

TABLE 7

Crossover percentage in male and female gametes. Family 4003.

REGION	NUMBER OF PLANTS	MALE GAMETES TESTED		FEMALE GAMETES TESTED		DIFFERENCE
		Total progeny	Crossover percentage	Total progeny	Crossover percentage	Crossover percentage
C-S_h	49	23,348	3.42±0.08	38,770	2.34±0.05	1.08±0.09
S_h-W_x	49	23,348	22.08±0.19	38,770	19.99±0.14	2.09±0.24
C-W_x	54	24,982	25.19±0.19	40,847	22.29±0.14	2.90±0.24

There is no question of the statistical significance of these differences, which are 8 to 12 times as large as their probable errors. The probability is inconceivably small that the total progenies representing male and female gametes are random samples from populations of gametes equal in crossover percentage. In both sections of the chromosome studied, the crossover percentage was distinctly higher in male gametes.

But we have seen that there are significant variations in crossing over between individual plants, and table 16 shows that there is great variation between plants, also, in relative frequency of crossovers in male and female gametes. It is possible that the difference in crossover percentage in the totalled data results from error of sampling the varying population of plants. In the method of comparison used above, the variation of plants is not a factor; the results are the same for any given crossover percentage and total number of grains, whether the plants making up the total are perfectly uniform or highly variable in crossing over.

The significance of the difference from this standpoint may be determined by averaging the differences in crossover percentage found in individual plants, and assigning to this average difference a probable error determined by BESSEL'S formula,

$$E = .6745\sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

Computed in this way the differences are as follows:

Region	*Number of plants*	*Mean difference in crossover percentage*
C-S_h	49	0.935 ± 0.115
S_h-W_x	49	2.065 ± 0.397
C-W_x	54	2.607 ± 0.375

Although the probable errors are considerably higher than in the preceding comparison, all three differences are distinctly significant. There is a high degree of probability, amounting to practical certainty, that an infinite number of plants of this population, tested under the same conditions, would have given a higher crossover percentage in male gametes than in female gametes, in both sections of the chromosome.

It does not follow, of course, that such a variation in crossing over is consistently associated with sex in maize. In fact, there are three good reasons for favoring the contrary view:

(1) Previously reported studies have given different results. Among the trials in which the same plants were used both as male and female parents, the only case in which a significant difference was found was one reported by EMERSON and HUTCHISON (1921), in which the crossover percentage in the C-S_h region was found significantly higher in *female* gametes. Other trials reported by EMERSON and HUTCHISON and by EYSTER (1922) showed no significant difference.

(2) Crossover variations analogous to those found in male and female gametes are found in different inflorescences of the same sex. For example, of the 21 plants of this family which produced both a main-stalk ear and a tiller ear, the crossover percentage (for C-W_x) was higher in the main-stalk ear in 17 cases. The mean of the differences in crossover percentage in the 21 plants was 2.26 ± 0.40, which is of the same order as the differences reported above for male and female gametes. It is probable that these differences result from the different environmental conditions under which the gametes develop in the main stalk and in the tiller, which is always several days later in flowering.[2] Since distinct differences in

[2] These data are presented and discussed in the next section of this paper.

crossover percentage are found in female gametes produced at different periods in the development of the plant, it is to be expected that crossover percentages may differ similarly in male and female gametes, for microsporogenesis and megasporogenesis also occur at different periods.

(3) The relative frequency of crossing over in the development of male and female gametes was found to differ consistently in different stocks. In addition to the data for family 4003, already reported, fairly extensive data are available in two other families, 4045 and 4070. Family 4045 was unrelated to family 4003, while family 4070 was related to both 4003 and 4045, its female parent being a full sib of that of 4003 and its male parent a full sib of that of 4045.

Two plantings of each of these families were made, the first on May 12 and the second on June 5. The later-planted series began to shed pollen about 10 days later than the earlier series. Thus the plants of the two series were likely to be under quite different weather conditions at any given stage of development. The crossover percentages for C-W_z, determined from the totalled progenies, are shown in table 8.

TABLE 8

Crossover percentage in male and female gametes. Families 4045 and 4070.

FAMILY	PLANTING	NUMBER OF PLANTS	MALE GAMETES TESTED		FEMALE GAMETES TESTED		DIFFERENCE CROSSOVER PERCENTAGE
			Total progeny	Crossover percentage	Total progeny	Crossover percentage	
4045	I	9	2745	25.36±0.56	3797	24.73±0.47	0.63±0.73
4045	II	5	1999	29.01±0.68	2045	27.38±0.66	1.63±0.95
4070	I	5	1668	26.26±0.73	2177	21.13±0.60	5.08±0.94
4070	II	10	2992	28.24±0.56	7284	24.71±0.34	3.53±0.65

Both plantings of 4045 gave an insignificant difference in crossover percentage in male and female gametes, while both plantings of 4070 gave a clearly significant difference in favor of the male gametes. Crossover percentages were higher in the later planting in both families, but increased in both male and female gametes, so that the relative frequency was not much affected.

VARIATION IN CROSSING OVER WITHIN THE INDIVIDUAL

To determine the extent to which crossing over in the development of male gametes might vary within the individual plant, successive pollinations were made from four plants at intervals of one or two days throughout the pollen-shedding season. The classified progenies of these plants

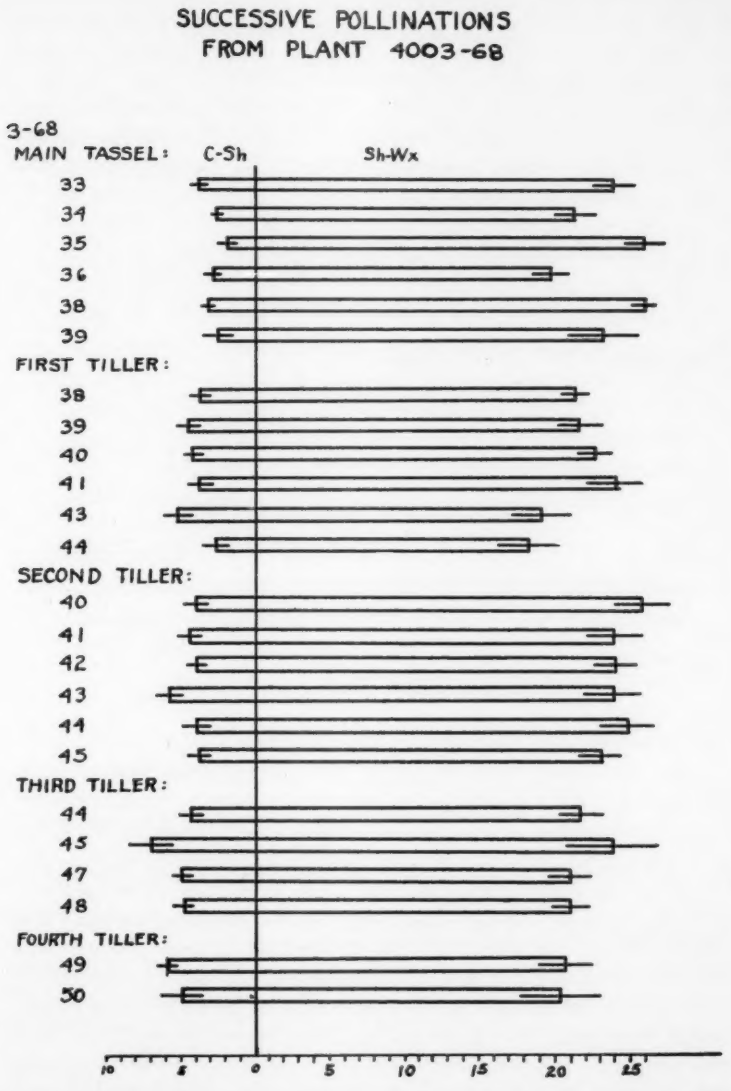

FIGURE 3.—Crossover percentages determined by repeated pollinations from plant 4003-68. Explanation in text.

are shown in tables 17 to 20, inclusive. The most extensive trial was made with plant 4003-68, from which pollinations were made daily, with two exceptions, through a period of 17 days. The plant produced 5 tassels, from each of which repeated pollinations were made. In all, 37 backcross ears were pollinated from this plant. The crossover percentages determined are shown graphically in figure 3. Similar but less extensive trials were made with plants 4013-2, 4017-5, and 4017-21. The results of these 3 trials are shown in figures 4 to 6, inclusive.

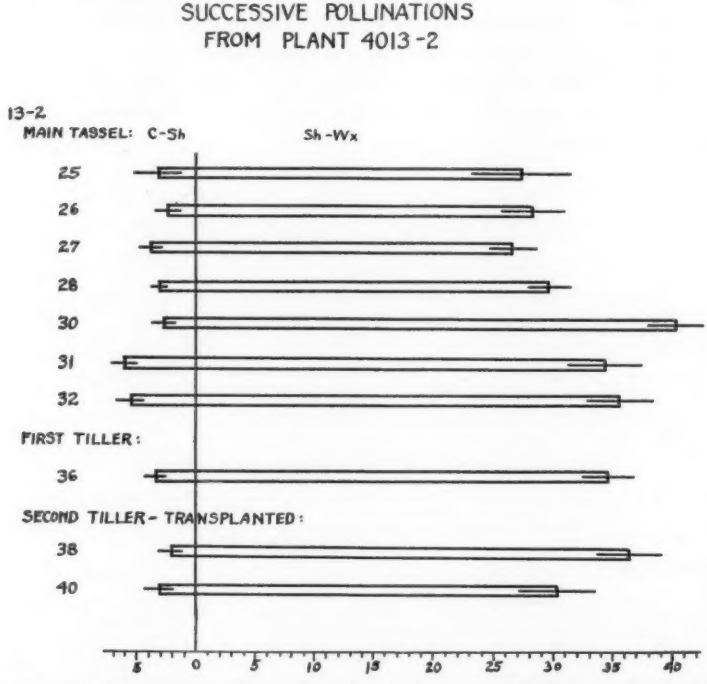

FIGURE 4.—Crossover percentages determined by repeated pollinations from plant 4013-2. Explanation in text.

In these graphs the crossover percentages for the C-S_h and S_h-W_x region are indicated by the length of the bars, while the probable error of each crossover determination is indicated by the length of the single line at the end of the bar. Thus, the first bar of figure 2 indicates crossover percentages of 3.7 ± 0.6 for C-S_h and 23.9 ± 1.4 for S_h-W_x. The dates given are continuous through July and August, July 31 being given as 31, August 1 as 32, etc.

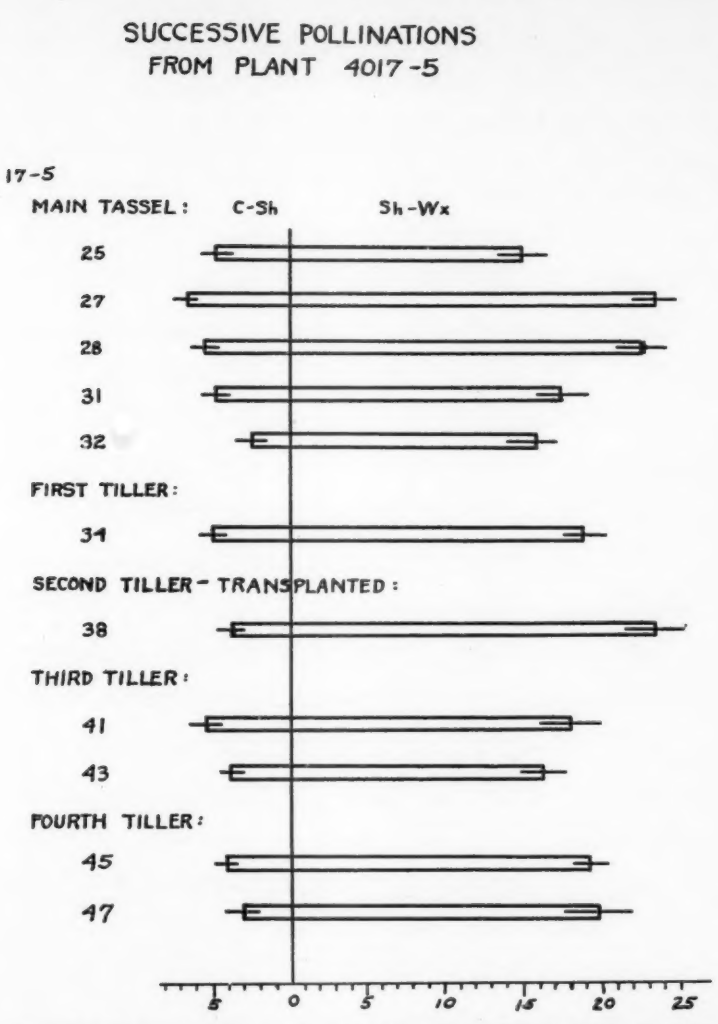

FIGURE 5.—Crossover percentages determined by repeated pollinations from plant 4017-5. Explanation in text.

The crossover percentages determined by successive pollinations from these 4 plants agree in indicating:

(1) There is no consistent increase or decrease in crossing over, with age;

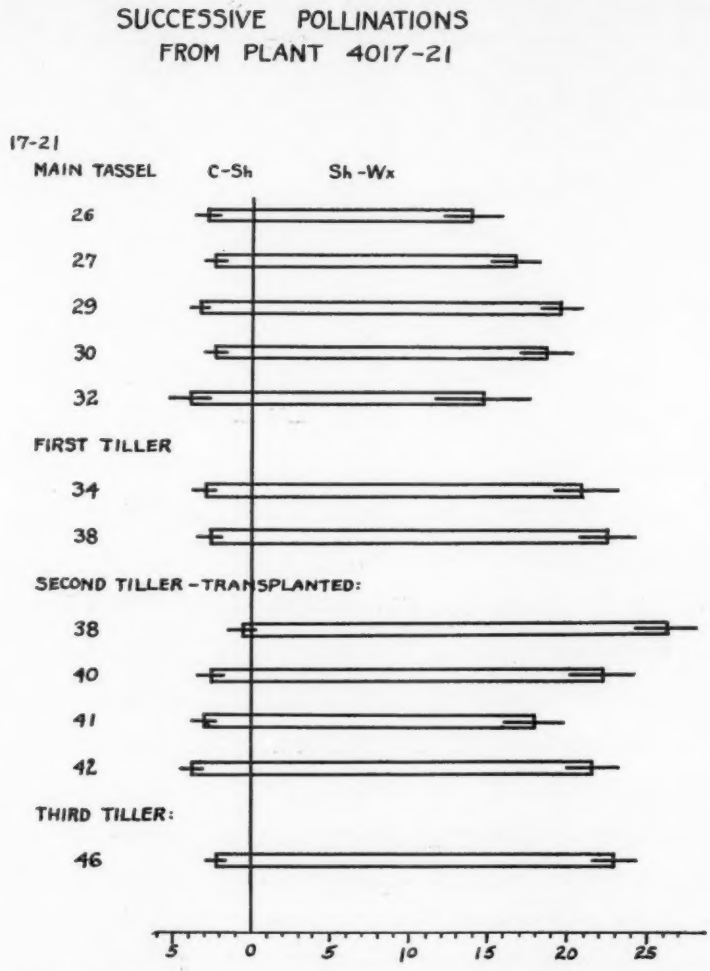

FIGURE 6.—Crossover percentages determined by repeated pollinations from plant 4017-21. Explanation in text.

(2) The fluctuations of the crossover percentage for the C-S_h region are not noticeably correlated, positively or negatively, with the fluctuations of the crossover percentage for the S_h-W_x region;

(3) The crossover percentages determined by pollinations from transplanted tillers were not significantly different from those of other tassels of the same plant; and

(4) The fluctuations in crossover percentage are surprisingly small when their probable errors are considered.

The possibility that these daily fluctuations in crossover percentage are due merely to error of sampling may be examined by the method which was used in studying the variation among different plants. In table 9 are shown the fluctuations from the mean, expected as a result of

TABLE 9

Daily variation in crossing over compared with variation expected as result of error of sampling. Repeated pollinations from 3-68, 13-2, 17-5 and 17-21.

PLANT	NUMBER OF TRIALS	OCCURRENCE	DEVIATIONS FROM MEAN, DIVIDED BY PROBABLE ERROR					
			1.0 or more	1.5 or more	2.0 or more	2.5 or more	3.0 or more	3.5 or more
3–68	24	*Expected*	*12.0*	*7.5*	*4.3*	*2.2*	*1.0*	*0.4*
		Observed:						
		C-S_h	12	10	4	1	0	0
		S_h-W_z	11	6	4	1	1	1
13–2	10	*Expected*	*5.0*	*3.1*	*1.8*	*0.9*	*0.4*	*0.2*
		Observed:						
		C-S_h	4	2	1	0	0	0
		S_h-W_z	8	3	2	2	1	1
17–5	12	*Expected*	*6.0*	*3.7*	*2.1*	*1.1*	*0.5*	*0.2*
		Observed:						
		C-S_h	6	4	3	2	2	1
		S_h-W_z	7	6	5	2	1	0
17–21	12	*Expected*	*6.0*	*3.7*	*2.1*	*1.1*	*0.5*	*0.2*
		Observed:						
		C-S_h	4	3	2	1	0	0
		S_h-W_z	7	5	3	1	1	0
Total....	58	*Expected*	*58.0*	*36.2*	*20.6*	*10.7*	*5.0*	*2.1*
		Observed:	59	39	24	10	6	3

error of sampling, compared with the fluctuations observed. There are, as would be expected, considerable divergences from the expected proportions within individual plants, but in the aggregate, the agreement of expected and observed fluctuations is very close. In other words, crossover percentages determined by repeated pollinations from a single homogeneous supply of pollen would be expected to vary almost as widely

as did the percentages determined by repeated pollinations throughout the pollen-shedding period.

Further evidence on the extent of variability within the individual plant is given by the crossover percentages determined by pollinations from main tassels and tiller tassels. In 19 plants of family 4003 crossover percentages were determined by pollination from both the main tassel and the first tiller tassel. These percentages are shown in table 10.

TABLE 10

Crossover percentage in main tassel and first tiller tassel. Family 4003.

PLANT NUMBER	FIRST TASSEL PERCENT	FIRST TILLER TASSEL PERCENT	DIFFERENCE PERCENT
13	19.4±2.1	30.4±2.2	11.0±3.1
16	26.9±1.7	30.6±1.8	3.7±2.5
18	23.6±1.4	26.0±1.7	2.4±2.2
23	27.0±2.0	26.7±1.5	−0.3±2.5
28	28.1±1.6	24.3±2.1	−3.8±2.6
41	29.5±1.8	26.1±2.2	−3.4±2.8
46	25.8±1.7	23.8±1.6	−2.0±2.3
54	24.8±1.4	25.7±2.2	0.9±2.6
55	27.4±1.7	21.9±1.9	−5.5±2.5
58	24.3±1.8	24.1±1.8	−0.2±2.5
61	25.3±1.9	26.3±1.7	1.0±2.5
65	27.3±2.5	29.7±1.6	2.4±3.0
66	19.8±1.9	22.0±1.5	2.2±2.4
68	27.5±1.4	25.0±1.3	−2.5±1.9
69	29.2±2.1	23.9±2.0	−5.3±2.9
73	25.2±2.4	21.8±2.4	−3.4±3.4
83	27.7±2.2	30.2±1.9	2.5±2.9
85	25.8±2.1	22.0±2.0	−3.8±2.9
99	29.1±1.5	26.1±1.7	−3.0±2.3

The tiller tassels do not differ consistently from the main tassels. In 8 cases the crossover value is higher in the main tassel; in 11 cases in the tiller tassel. Of the 19 differences, 11 are as great as their probable errors, 2 are twice as great, and 1 is three times as great,—again only slightly more variability than would be expected to result from error of sampling. The mean difference in crossover percentage is 0.37 ± 0.60, in favor of the main tassel. The difference is less than its probable error and of course has no significance.

Thus, in this material, crossing over in the production of male gametes has been a relatively constant character of the individual plant. Any environmental variation which has occurred has been too slight to be detected in populations of the size used in this study. Fortunately, the

material used will permit a considerable increase in precision. By using large-eared recessive stocks and pollinating as many ears as possible from each sack of pollen taken from the heterozygous plant, it is possible to reduce the probable error of the crossover percentages representing each day's pollination, at least to 0.2 for the C-S_h region and 0.4 for the C-W_z or S_h-W_z regions. Fluctuations of 1 or 2 crossover units would then be clearly significant.

While crossing over in the production of male gametes varied little within the plant, it varied widely in different plants. The four plants from which daily pollinations were made are favorable material for this comparison, for their large total progenies establish their crossover percentages with a fairly high degree of precision, while the repeated pollinations demonstrate that daily fluctuations were a very minor factor in the differences. These four plants represent three families, of which two were very closely related. Family 4017 was produced by crossing two plants which were full sibs of the parents of family 4003. Family 4013 was not related to the other two families.

The crossover percentages determined from the totalled progenies of the four plants are shown in table 11.

TABLE 11

Individual differences in crossing over.

PLANT	MALE GAMETES			FEMALE GAMETES
	C-S_h	S_h-W_z	C-W_z	C-W_z
3–68	3.9±0.1	22.8±0.3	26.4±0.3	25.8±0.9
13–2	3.3±0.3	30.3±0.7	33.2±0.7	28.7±1.1
17–5	4.3±0.2	19.0±0.5	23.0±0.5	19.6±0.8
17–21	2.9±0.2	20.8±0.5	23.6±0.5	21.0±1.0

There are significant differences in crossing over between any two of the four plants compared. The most striking difference is between 4013-2 and 4017-5, which, as it happened, were planted on the same day, reached pollen-shedding on the same day, and grew under uniform conditions within 5 yards of one another. Plant 17-5, which had a much lower crossover percentage in the S_h-W_z region, had a somewhat higher crossover percentage in the C-S_h region. The latter difference is perhaps insignificant, but there can be no doubt of the significance of the differences in the S_h-W_z region or in the C-W_z region taken as a whole, which are, respectively, 11.3 ± 0.9 and 10.2 ± 0.9. A similar difference in crossover percentage was found in the ears of these two plants, in which the differ-

ence in the crossover percentage for C-W_x was 9.1 ± 1.4. Unfortunately, separations for S_h s_h could not be made in the ears of plant 17-5, so crossover percentages in female gametes could not be determined for the C-S_h and S_h-W_x sections. For the C-W_x region, considered as a whole, crossing over was about 45 percent more frequent in plant 13-2, in the development of both male and female gametes.

In female gametes the crossover percentages in main stalks and tillers showed a relation very different from that found in male gametes. Of the 70 plants for which crossover percentages for C-W_x were determined, 21 produced a backcrossed ear on both the main stalk and the first tiller. The crossover percentages for these ears are shown in table 12.

TABLE 12

Crossover percentage in first ear and first tiller ear. Family 4003.

PLANT	FIRST EAR PERCENT	FIRST TILLER EAR PERCENT	DIFFERENCE PERCENT
7	24.3±1.3	27.0±1.3	2.7±1.8
11	22.6±1.3	26.2±1.4	3.6±1.9
13	20.5±1.5	22.5±1.6	2.0±2.2
16	19.8±1.4	20.9±1.2	1.1±1.8
17	20.5±1.3	24.1±1.3	3.6±1.8
19	20.5±1.6	17.7±2.2	−2.8±2.7
24	17.8±1.3	26.9±1.4	9.1±1.9
31	22.5±1.3	22.7±1.5	0.2±2.0
33	20.3±1.2	18.9±1.2	−1.4±1.7
34	23.1±1.2	25.0±2.1	1.9±2.4
46	25.5±1.3	26.6±1.3	1.1±1.8
52	22.2±1.3	22.0±1.3	−0.2±1.8
53	17.8±1.4	23.6±1.4	5.8±2.0
54	17.5±1.2	17.8±1.3	0.3±1.8
63	25.6±1.2	29.2±1.3	3.6±1.8
67	21.0±1.5	24.2±1.3	3.2±2.0
68	26.4±1.3	25.3±1.2	−1.1±1.8
69	22.9±1.2	25.7±1.4	2.8±1.8
78	27.6±2.3	28.8±1.4	1.2±2.7
85	17.3±1.5	22.4±1.6	5.1±2.2
99	20.0±1.3	25.7±1.3	5.7±1.8

The crossover percentages are consistently higher in the tiller ears. In only 4 of the 21 cases is the main-stalk ear higher in crossover percentage, and only one of these differences is as great as its probable error. The mean of the differences is 2.26 ± 0.40, or 5.65 times its probable error. Similarly, the crossover percentages determined from the totalled progenies are 21.26 ± 0.29 for the main-stalk ear and 23.96 ± 0.31 for the tiller ear, giving a difference in favor of the tiller ear of 2.70 ± 0.42. Thus,

different ears of the same plant have undoubtedly differed significantly in crossover percentage.

Whether this difference is due to age or to different environmental conditions at a critical period cannot be certainly determined from the available evidence. Whatever the cause of the difference between inflorescences of main and tiller stalks, however, it is clear that crossing over was far more affected by it in female than in male gamete development.

The first and second ears of the main stalk were strikingly similar in crossover percentage, in the 52 plants which produced two main-stalk ears. In this family the two ears of the main stalk were usually of about the same size and silked on the same day. Presumably, they developed under practically the same environmental conditions. The differences between their crossover percentages were no greater than those to be expected from fluctuations of sampling, as shown in table 13.

TABLE 13

Differences in crossover percentage in first and second ear compared with differences expected from error of sampling.

	NUMBER OF PLANTS	DIFFERENCE, DIVIDED BY PROBABLE ERROR		
		1.0 or more	1.0 or more	3.0 or more
Expected	*47*	*23.50*	*8.33*	*2.02*
Observed:				
C-S_h	47	23	8	0
S_h-W_x	47	20	7	0
Expected	*52*	*26.00*	*9.22*	*2.24*
Observed:				
C-W_x	52	25	6	1

SUMMARY

Among a maize population of full sibs grown under uniform conditions:

(1) Crossing over was found only moderately variable, when due allowance was made for fluctuations of sampling.

(2) Crossover percentages determined for the short C-S_h region were much more variable than those for the long S_h-W_x region. This is probably due to the fact that the former are much more greatly affected by errors of sampling.

(3) Crossover percentages in female gametes were more variable than crossover percentages in male gametes.

(4) Crossover percentages in the first and second ear of the main stalk were highly correlated.

(5) Crossover variations in male gametes were not correlated with plant height, number of ears, or number of tillers, but were possibly correlated, negatively, to a slight extent, with date of first pollen.

Crossover variations in female gametes were correlated negatively with plant height, number of ears, and possibly number of tillers, but were not correlated with date of first pollen.

(6) Crossover percentages in male and female gametes of the same plants were not significantly correlated.

(7) Crossover percentages were significantly higher in male gametes than in female gametes of the same plants.

Within the individual plant:

(8) Crossover percentages in male gametes, determined by repeated pollinations throughout the pollen-shedding season, did not differ significantly.

(9) Pollen of transplanted tillers did not differ significantly in crossover percentage from pollen of other tassels of the same plant.

(10) Crossover percentages in female gametes were significantly higher in tiller ears than in ears of the main stalk. In male gametes no such difference was found. Thus, crossing over in the development of female gametes was apparently affected by age or environmental conditions which had no distinct effect on crossing over in the development of male gametes.

Among plants of different families:

(11) A single random comparison of two plants from unrelated families showed a wide and clearly significant difference in crossing over. The differences were similar in male and female gametes.

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Appendix—Tables 14 to 22

Table 14

Crossover data, male gametes, family 4003.

Plant number	Non-crossovers		Crossovers in region 1		Crossovers in region 2		Crossovers in regions 1, 2		Total
3	136	115	1	2	33	34	0	0	321
5	59	84	0	4	22	15	1	0	185
7	242	264	13	12	68	64	0	2	665
10	131	109	4	3	33	25	1	0	306
11	245	203	10	8	51	48	2	3	570
12	104	100	5	1	47	26	1	0	284
13	223	211	8	10	63	68	0	0	583
16	186	216	10	7	75	69	0	0	563
18	309	290	16	22	66	87	1	1	792
19	102	95	9	6	37	26	0	0	275
20	81	91	4	1	36	27	0	0	240
23	183	247	13	6	78	63	0	1	591
24	101	105	1	0	13	20	0	0	240
27	101	101	5	2	29	27	0	0	265
28	175	208	5	8	72	55	1	0	524
29	150	148	4	1	43	36	0	1	383
30	178	165	11	5	40	59	0	2	460
33	415	436	14	9	114	114	0	0	1102
34	73	53	3	4	15	18	0	0	166
35	122	114	7	6	32	32	0	1	314
40	92	82	6	3	19	15	0	0	217
41	164	157	9	6	49	62	0	0	447
44	62	74	1	3	26	27	0	0	193
46	209	259	18	14	68	53	1	0	622
47	74	77	2	2	29	25	0	0	209
52	279	296	8	9	91	88	1	1	773
53	121	108	4	4	37	41	0	0	315
54	232	250	10	4	81	66	0	0	643
55	307	291	18	19	76	81	0	1	793
58	209	194	5	11	51	62	0	1	533
60	171	170	16	13	43	44	0	0	457
61	285	301	9	12	83	99	0	1	790
63	78	100	7	2	21	25	0	0	233
65	156	178	8	8	62	58	0	0	470
66	250	228	14	12	54	48	0	0	606
68	669	703	45	32	209	213	1	1	1873
69	268	264	10	12	79	84	0	1	718
70	91	77	3	4	39	37	0	0	251
71	308	278	15	2	78	94	2	0	777
72	143	132	5	7	46	41	0	1	375
73	129	101	2	5	30	34	2	0	303
74	81	131	5	1	26	26	0	0	270
75	196	189	13	6	74	55	1	0	534

TABLE 14 (continued)

PLANT NUMBER	NON-CROSSOVERS	CROSSOVERS IN REGION				TOTAL
		1		2	1, 2	
76	85 111	5 4		28 30	0 0	263
78	97 104	4 5		27 31	0 0	268
80	217 241	10 6		59 67	0 0	600
81	97 80	4 2		18 25	0 0	226
82	135 136	2 4		35 53	0 0	365
83	242 248	11 11		99 64	0 0	675
85	145 161	7 10		39 41	1 4	408
87	96 107	5 2		29 34	0 0	273
88	86 77	7 4		21 21	0 0	216
89	54 45	1 2		13 12	0 0	127
90	219 218	8 9		60 60	0 0	574
92	130 123	10 3		44 32	0 0	342
95	37 43	4 1		12 12	0 0	109
96	73 67	3 3		36 25	0 0	207
98	170 153	8 9		46 49	0 0	435
99	235 261	8 14		81 88	0 1	688
Total	20,178	870		5919	39	27,006

TABLE 15

Crossover data, female gametes, family 4003.
(Tiller ears excluded).

PLANT	NON-CROSSOVERS		CROSSOVERS IN REGION						TOTAL
			1		2		1, 2		
2	116	96	8	4	24	41	3	2	294
4	187	161	5	4	41	41	1	1	441
5	337	333	10	12	74	72	4	2	844
7	342	324	17	13	99	96	0	0	891
9	307	296	5	6	100	102	1	0	817
10	210	205	6	5	52	33	1	0	512
11	185	178	1	4	40	61	0	0	469
13	292	312	4	6	65	92	0	0	771
15	161	200	6	2	46	61	0	0	476
16	390	360	6	5	65	83	1	1	911
17	206	155	2	4	36	51	0	0	454
18	254	235	6	7	62	77	0	0	641
19	188	221	5	8	55	53	0	0	530
20	331	330	10	9	84	81	1	1	847
24	216	183	3	2	47	38	0	0	489
27	316	328	11	6	109	107	0	0	877
28	464	471	15	15	105	126	0	0	1196
29	448	470	5	8	100	96	0	0	1127
30	262	247	4	3	61	63	0	0	640
31	273	271	7	9	73	79	0	1	713
32	250	258	8	9	50	52	0	0	627
33	221	218	7	6	48	51	0	0	551
34	362	355	11	17	95	73	2	0	915
40	385	377	10	14	89	86	0	0	961
44	406	386	7	11	90	92	0	0	992
46	354	345	14	14	117	112	0	1	957
47	325	335	14	19	102	94	0	0	889
52	358	344	7	15	92	94	0	0	910
53	179	162	4	7	31	34	0	0	417
54	406	459	8	11	93	82	0	0	1059
55	182	160	8	10	50	61	0	0	471
58	70	79	6	1	12	18	0	0	186
59	419	425	17	15	80	92	0	1	1049
61	410	426	3	5	89	91	0	0	1024
63	338	328	12	16	87	86	0	0	867
65	296	277	4	3	60	61	1	0	702
66	393	391	15	15	89	96	0	0	999
67	212	201	5	5	46	56	0	0	525
68	157	168	8	8	50	53	0	0	444
69	206	198	5	5	58	53	0	0	525
70	307	315	18	15	114	104	1	1	875
71	439	424	12	18	87	91	0	0	1071
72	469	419	13	18	106	92	0	0	1117
73	305	277	16	11	96	99	0	0	804

TABLE 15 (continued)

PLANT	NON-CROSSOVERS	CROSSOVERS IN REGION			TOTAL
		1	2	1, 2	
75	268 277	9 9	121 103	1 1	789
76	456 379	22 17	98 104	0 0	1076
77	218 244	9 4	67 75	0 0	617
80	301 274	10 6	60 68	0 0	719
81	338 364	11 6	95 97	0 0	911
83	262 247	4 0	71 67	0 0	651
85	337 331	13 11	45 81	1 0	819
86	262 279	2 4	50 45	0 0	642
87	325 380	9 7	77 102	0 0	900
88	404 403	8 7	130 136	0 0	1088
89	306 297	9 8	93 88	0 0	801
90	207 206	5 6	61 66	1 0	552
93	119 107	1 0	16 31	0 0	274
95	157 155	4 6	44 42	1 0	409
96	348 360	9 8	80 98	0 0	903
98	278 266	4 9	67 67	0 0	691
99	389 375	11 13	97 95	0 0	980
100	50 52	1 3	14 13	0 0	133
Total	35,658	1033	9109	32	45,832

Ears without separation of S_h s_h (crossovers in C-W_x region)

3	157 148	— —	44 61	— —	410
12	95 99	— —	41 39	— —	274
14	307 304	— —	118 99	— —	828
35	97 108	— —	30 29	— —	264
41	384 362	— —	83 110	— —	939
42	98 89	— —	26 20	— —	233
78	74 65	— —	33 18	— —	190
Grand total	38,045	——	10,893	——	48,970

TABLE 16

Crossover percentage in male and female gametes. Family 4003.

PLANT NUMBER	C-S_h REGION			S_h-W_z REGION			C-W_z REGION		
	Male gametes	Female gametes	Difference	Male gametes	Female gametes	Difference	Male gametes	Female gametes	Difference
5	2.7±0.9	3.3±0.3	−0.6±0.9	20.5±2.1	18.0±1.0	2.5±2.3	22.2±2.2	19.9±1.0	2.3±2.4
7	4.1±0.5	3.4±0.3	0.7±0.6	20.2±1.1	21.9±0.9	−1.7±1.4	23.6±1.1	25.3±1.0	−1.7±1.5
10	2.6±0.7	2.3±0.4	0.3±0.8	19.3±1.6	16.8±1.2	2.5±2.0	21.2±1.7	18.8±1.3	2.4±2.1
11	4.0±0.5	1.1±0.5	2.9±0.7	18.2±1.2	21.5±1.3	−3.3±1.8	20.5±1.2	22.6±1.3	−2.1±1.8
13	3.1±0.5	1.3±0.4	1.8±0.7	22.5±1.1	20.4±1.0	2.1±1.5	25.6±1.2	21.7±1.0	3.9±1.6
16	3.0±0.5	1.4±0.3	1.6±0.6	25.6±1.2	16.5±0.9	9.1±1.5	28.6±1.2	17.5±1.0	11.1±1.6
18	5.1±0.4	2.0±0.4	3.1±0.6	19.6±1.0	21.7±1.1	−2.1±1.5	24.1±1.0	23.7±1.1	0.4±1.5
19	5.5±0.7	2.5±0.4	3.0±0.8	22.9±1.7	20.4±1.2	2.5±2.1	28.4±1.8	22.8±1.3	5.6±2.2
20	2.1±0.8	2.5±0.3	−0.4±0.9	26.3±1.8	19.7±1.0	6.6±2.1	28.3±1.9	21.7±1.0	6.6±2.1
24	0.4±0.8	1.0±0.5	−0.6±0.9	13.8±1.8	17.4±1.3	−3.6±2.2	14.2±1.9	18.4±1.3	−4.2±2.3
27	2.6±0.7	1.9±0.3	0.7±0.8	21.1±1.7	24.6±0.9	−3.5±1.9	23.8±1.8	26.6±1.0	−2.8±2.1
28	2.7±0.5	2.5±0.3	0.2±0.6	24.4±1.2	19.3±0.8	5.1±1.4	26.7±1.3	21.8±0.8	4.9±1.5
29	1.6±0.6	1.2±0.3	0.4±0.7	20.9±1.4	17.4±0.8	3.5±1.6	21.9±1.5	18.5±0.9	3.4±1.7
30	3.9±0.6	1.1±0.4	2.8±0.7	22.0±1.3	19.4±1.1	2.6±1.7	25.0±1.4	20.5±1.1	4.5±1.8
33	2.1±0.4	2.4±0.4	−0.3±0.6	20.7±0.8	18.0±1.2	2.7±1.4	22.8±0.9	20.3±1.2	2.5±1.5
34	4.2±0.9	3.3±0.3	0.9±0.9	19.9±2.2	18.6±0.9	1.3±2.4	24.1±2.3	21.4±1.0	2.7±2.5
40	4.1±0.8	2.5±0.3	1.6±0.9	15.7±1.9	18.2±0.9	−2.5±2.1	19.8±2.0	20.7±0.9	−0.9±2.2
44	2.1±0.9	1.8±0.3	0.3±0.9	27.5±2.0	18.3±0.9	9.2±2.2	29.5±2.1	20.2±0.9	9.3±2.3
46	5.3±0.5	3.0±0.3	2.3±0.6	19.6±1.1	24.0±0.9	−4.4±1.4	24.6±1.2	26.9±0.9	−2.3±1.5
47	1.9±0.8	3.7±0.3	−1.8±0.9	25.8±2.0	22.0±0.9	3.8±2.2	27.8±2.0	25.8±1.0	2.0±2.2
52	2.5±0.4	2.4±0.3	0.1±0.5	23.4±1.0	20.4±0.9	3.0±1.3	25.4±1.1	22.9±1.0	2.5±1.5
53	2.5±0.7	2.6±0.5	−0.1±0.9	24.8±0.6	15.6±1.4	9.2±1.5	27.3±1.7	18.2±1.4	9.1±2.2
54	2.2±0.5	1.8±0.3	0.4±0.6	22.9±1.1	16.5±0.9	6.4±1.4	25.0±1.2	18.3±0.9	6.7±1.5
55	4.8±0.4	3.8±0.5	1.0±0.7	19.9±1.0	23.6±1.3	−3.7±1.6	24.5±1.0	27.4±1.3	−2.9±1.6

TABLE 16 (continued)

PLANT NUMBER	C-S_h REGION			S_h-W_x REGION			C-W_x REGION		
	Male gametes	Female gametes	Difference	Male gametes	Female gametes	Difference	Male gametes	Female gametes	Difference
58	3.2±0.5	3.8±0.7	−0.6±0.9	21.4±1.2	16.1±2.1	5.3±2.4	24.2±1.3	19.9±2.1	4.3±2.5
61	2.8±0.4	0.8±0.3	2.0±0.5	23.2±1.0	17.6±0.9	5.6±1.3	25.7±1.1	18.4±0.9	7.3±1.4
63	3.9±0.8	3.2±0.3	0.7±0.9	19.7±1.9	20.0±1.0	−0.3±2.1	23.6±1.9	23.2±1.0	0.4±2.1
65	3.4±0.6	1.1±0.4	2.3±0.7	25.5±1.3	17.4±1.1	8.1±1.7	28.9±1.4	18.2±1.1	10.7±1.8
66	4.3±0.5	3.0±0.3	1.3±0.6	16.8±1.2	18.5±0.9	−1.7±1.5	21.1±1.2	21.5±0.9	−0.4±1.5
68	4.2±0.3	3.6±0.5	0.6±0.6	22.6±0.7	23.2±1.3	−0.6±1.5	26.6±0.7	26.8±1.4	−0.2±1.6
69	3.2±0.4	1.9±0.4	1.3±0.6	22.8±1.1	21.1±1.2	1.7±1.6	25.8±1.1	23.0±1.3	2.8±1.7
70	2.8±0.8	4.0±0.3	−1.2±0.9	30.3±1.8	25.1±0.9	5.2±2.0	33.1±1.9	28.7±1.0	4.4±2.1
71	2.7±0.4	2.8±0.3	−0.1±0.5	24.6±1.0	16.6±0.9	8.0±1.3	26.8±1.1	19.4±0.9	7.4±1.4
72	3.5±0.6	2.8±0.3	0.7±0.7	23.5±1.5	17.7±0.8	5.8±1.7	26.4±1.5	20.5±0.9	5.9±1.7
73	3.0±0.7	3.4±0.4	−0.4±0.8	21.8±1.6	24.3±1.0	−2.5±1.9	23.4±1.7	27.6±1.0	−4.2±2.0
75	3.7±0.5	2.5±0.4	1.2±0.7	24.3±1.2	28.6±1.0	−4.3±1.6	27.7±1.3	30.7±1.0	−3.0±1.6
76	3.4±0.7	3.6±0.3	−0.2±0.8	22.1±1.7	18.8±0.9	3.3±1.9	25.5±1.8	22.4±0.9	3.1±2.0
80	2.7±0.5	2.2±0.4	0.5±0.7	21.0±1.2	17.8±1.0	3.2±1.6	23.7±1.2	20.0±1.1	3.7±1.6
81	2.7±0.3	1.9±0.3	0.8±0.4	19.0±1.9	21.1±0.9	−2.1±2.1	21.7±2.0	22.9±1.0	−1.2±2.2
83	3.3±0.5	0.6±0.4	2.7±0.7	24.1±1.1	21.2±1.1	2.9±1.6	27.4±1.1	21.8±1.1	5.6±1.6
85	5.4±0.6	2.9±0.4	2.5±0.7	20.8±1.4	15.5±1.0	5.3±1.7	23.8±1.5	18.3±1.0	5.5±1.8
87	2.6±0.7	1.8±0.3	0.8±0.8	23.1±1.7	19.9±0.9	3.2±1.9	25.6±1.8	21.7±1.0	3.9±2.1
88	5.1±0.8	1.4±0.3	3.7±0.9	19.4±1.9	24.4±0.8	−5.0±2.1	24.5±2.0	25.8±0.9	−1.3±2.2
89	2.4±1.1	2.1±0.4	0.3±1.2	19.7±2.5	22.6±1.0	−2.9±2.7	22.0±2.6	24.7±1.0	−2.7±2.8
90	3.0±0.5	2.2±0.4	0.8±0.7	20.9±1.2	23.2±1.2	−2.3±1.7	23.9±1.2	25.0±1.2	−1.1±1.7
95	4.6±1.1	2.7±0.5	1.9±1.2	22.0±2.7	21.3±1.4	0.7±3.0	26.6±2.8	23.5±1.4	3.1±3.1
96	2.9±0.8	1.9±0.3	1.0±0.9	29.5±2.0	19.7±0.9	9.8±2.2	32.4±2.1	21.6±1.0	10.8±2.3
98	3.9±0.6	1.9±0.4	2.0±0.7	21.8±1.4	19.4±1.1	2.4±1.8	25.7±1.4	21.3±1.1	4.4±1.8
99	3.3±0.5	2.4±0.3	0.9±0.6	24.7±1.1	19.6±0.9	5.1±1.4	27.8±1.1	22.0±0.9	5.8±1.4

TABLE 17

Repeated pollinations from plant 4003-68.

TASSEL	DATE AUGUST	NON-CROSSOVER		CROSSOVERS IN REGION						TOTAL
				1		2		1, 2		
Main	2	140	176	8	8	57	47	0	0	436
	4	135	182	4	7	46	42	0	0	416
	4	151	140	4	2	56	47	0	1	401
	5	182	205	10	4	40	58	0	0	499
	7	297	341	19	10	119	115	0	0	901
	8	42	51	0	2	15	14	0	0	124
First tiller	7	184	194	11	7	51	57	0	0	504
	8	111	101	5	8	27	35	0	0	287
	9	268	253	20	10	81	81	0	0	713
	10	77	93	4	5	30	27	0	0	236
	12	70	76	6	4	20	17	0	0	193
	13	74	82	2	2	14	21	0	1	196
Second tiller	9	96	75	6	4	25	38	0	0	244
	10	83	93	6	3	23	33	1	1	243
	11	138	143	6	7	43	48	1	1	387
	12	82	90	6	8	25	33	0	0	244
	13	68	82	5	3	28	24	0	0	210
	14	128	155	7	6	35	53	0	1	385
Third tiller	13	143	141	9	7	48	34	0	1	383
	14	26	32	3	3	8	12	0	0	84
	16	134	138	10	6	26	48	0	2	364
	17	155	171	12	9	51	41	0	0	439
Fourth tiller	18	106	117	11	6	28	37	1	0	306
	19	35	38	4	1	14	6	0	0	98

TABLE 18

Repeated pollinations from plant 4013-2.

TASSEL	DATE	NON-CROSSOVER	CROSSOVERS IN REGION						TOTAL
			1		2		1, 2		
Main	July 25	23	24	0	2	9	8	0	0	66
	July 27	86	96	5	4	30	34	0	0	255
	July 28	153	153	8	4	51	72	0	1	442
	July 29	49	57	2	2	16	23	1	0	150
	July 30	57	58	3	2	30	44	0	0	194
	July 31	41	35	3	4	18	22	0	0	123
	Aug. 1	34	48	3	4	18	27	0	0	134
First tiller	Aug. 5	76	59	4	3	35	34	0	0	211
Second tiller (transplanted)	Aug. 7	49	50	2	0	22	30	0	1	154
	Aug. 9	34	39	1	1	13	16	1	0	105

TABLE 19

Repeated pollinations from plant 4017-5.

TASSEL	DATE	NON-CROSSOVER	CROSSOVERS IN REGION						TOTAL
			1		2		1, 2		
Main	July 25	130	103	7	6	18	24	0	0	288
	July 27	116	121	9	12	39	39	0	1	337
	July 28	115	106	1	16	36	34	0	0	308
	July 31	99	102	9	3	31	14	0	0	258
	Aug. 2	96	107	2	4	24	15	0	0	248
First tiller	Aug. 3	152	145	12	7	35	38	0	0	389
Second tiller (transplanted)	Aug. 7	90	73	5	2	25	26	1	0	222
	Aug. 10	76	64	0	0	14	19	0	1	174
Third tiller	Aug. 10	77	66	4	4	21	10	0	2	184
	Aug. 12	142	151	9	5	32	27	0	0	366
Fourth tiller	Aug. 14	211	176	6	15	55	41	0	0	504
	Aug. 16	67	56	4	1	20	11	0	0	159

TABLE 20

Repeated pollinations from plant 4017-21.

TASSEL	DATE	NON-CROSSOVER	CROSSOVERS IN REGION			TOTAL
			1	2	1, 2	
Main	July 26	82 86	1 5	20 14	0 0	208
	July 27	127 106	2 5	29 21	0 0	290
	July 29	137 146	7 9	32 44	0 0	375
	July 30	94 102	3 3	26 22	0 0	250
	Aug. 2	27 32	2 1	7 4	0 0	73
First tiller	Aug. 3	83 91	4 3	26 24	0 0	231
	Aug. 7	77 87	4 1	25 25	0 1	220
Second tiller (transplanted)	Aug. 7	54 66	0 1	19 26	0 0	166
	Aug. 9	57 57	3 1	18 17	0 0	153
	Aug. 10	75 74	2 4	16 19	0 0	190
	Aug. 11	106 124	9 3	36 33	0 0	311
Third tiller	Aug. 16	106 150	3 4	31 49	0 1	344

TABLE 21

Crossover data, first tassel and first tiller tassel, family 4003.

PLANT	FIRST TASSEL					FIRST TILLER TASSEL				
	Non-crossover	Crossovers in region			Total	Non-crossover	Crossovers in region			Total
		1	2	1, 2			1	2	1, 2	
13	88 70	2 2	16 18	0 0	196	66 62	3 3	22 28	0 0	184
16	112 111	5 4	40 33	0 0	305	74 105	5 3	35 36	0 0	258
18	181 185	12 12	34 51	1 0	476	128 105	4 10	32 36	0 1	316
23	69 85	6 2	27 22	0 0	211	114 162	7 4	51 41	0 1	380
28	110 130	4 5	51 34	1 0	335	65 78	1 3	21 21	0 0	189
41	90 101	5 3	29 43	0 0	271	74 56	4 3	20 19	0 0	176
46	94 121	12 7	27 29	1 0	291	115 138	6 7	41 24	0 0	331
54	165 187	7 2	66 41	0 0	468	67 63	3 2	15 25	0 0	175
55	114 117	10 9	35 33	0 0	318	95 94	5 7	18 23	0 0	242
58	106 99	2 4	25 35	0 1	272	103 95	3 7	26 27	0 0	261
61	80 103	4 2	29 27	0 0	245	107 102	3 4	31 37	0 1	285
65	48 56	1 2	21 15	0 0	143	108 122	7 6	41 43	0 0	327
66	101 89	4 2	23 18	0 0	237	149 139	10 10	31 30	0 0	369
68	140 176	8 8	57 47	0 0	436	184 194	11 7	51 57	0 0	504
69	66 70	3 4	22 27	0 0	192	88 84	2 4	24 24	0 0	226
73	60 49	1 2	17 17	1 0	147	69 52	1 3	13 17	1 0	156
83	67 61	2 2	26 19	0 0	177	85 86	5 6	40 23	0 0	245
85	67 74	3 3	25 18	0 0	190	78 87	4 7	14 23	1 4	218
99	127 144	6 5	50 50	0 0	382	108 117	2 9	31 38	0 1	306
Total	3913	177	1197	5	5292	3818	191	1129	10	5148

TABLE 22

Crossover data, first ear and first tiller ear, family 4003.

PLANT	FIRST EAR					FIRST TILLER EAR				
	Non-crossovers	Crossovers in region			Total	Non-crossovers	Crossovers in region			Total
		1	2	1, 2			1	2	1, 2	
7	195 173	8 5	55 49	0 0	485	181 159	7 7	56 61	0 0	471
11	185 178	1 4	40 61	0 0	469	163 146	5 6	40 53	0 0	413
13	130 157	1 1	34 38	0 0	361	118 127	3 5	34 29	0 0	316
16	162 159	2 1	36 40	0 0	400	219 204	3 1	53 55	0 0	535
17	206 155	2 4	36 51	0 0	454	157 171	3 6	37 55	0 0	429
19	106 123	4 6	25 24	0 0	288	61 69	0 0	12 16	0 0	158
24	216 183	3 2	47 38	0 0	489	165 139	3 3	58 49	0 0	417
31	172 165	3 7	43 45	0 0	435	132 149	4 3	35 43	0 0	366
33	221 218	7 6	48 51	0 0	551	218 227	1 3	44 56	0 0	549
34	198 179	6 9	58 43	2 0	495	59 73	2 2	27 13	0 0	176
46	191 167	7 5	50 61	0 1	482	175 164	5 6	59 53	0 0	462
52	171 173	3 9	41 45	0 0	442	187 165	0 2	40 57	0 0	451
53	179 162	4 7	31 34	0 0	417	158 140	3 1	39 48	0 0	389
54	229 233	4 6	42 46	0 0	560	196 195	7 5	38 37	0 0	478
61	216 234	2 2	47 43	0 0	544	47 66	0 3	12 17	0 0	145
63	182 187	8 8	54 52	0 0	491	160 142	9 12	52 53	0 0	428
67	146 128	4 3	33 33	0 0	347	175 191	3 4	63 48	0 0	484
68	157 168	8 8	50 53	0 0	444	216 214	13 14	67 62	0 1	587
69	206 198	5 5	58 53	0 0	525	144 165	7 7	41 52	0 0	416
80	122 110	5 3	25 26	0 0	291	54 53	1 2	19 17	0 0	146
85	137 135	6 5	16 30	0 0	329	131 111	3 7	30 31	0 0	313
99	198 185	4 6	43 42	0 0	478	158 161	2 9	49 48	1 0	428
Total	7695	209	1870	3	9777	6504	192	1858	2	8557

EFFECT OF THE WAXY GENE IN MAIZE POLLEN,— A REPLY TO CRITICISMS[1]

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It was independently discovered and coincidentally reported by DEMEREC (1924) and by BRINK and MACGILLIVRAY (1924) that maize pollen grains carrying the waxy gene give a unique reaction with the iodine-potassium-iodide solution commonly employed in detecting starch. LONGLEY (1924) has also reported this phenomenon. In a recent paper, KIESSELBACH and PETERSEN (1925) question the validity of our observations and conclusions.

In our original papers we showed that pollen of flint, dent, pop and sweet varieties of maize contain reserves giving the well-known blue reaction characteristic of starch when tested with iodine. On the other hand, the contents of pollen grains produced by homozygous waxy plants fail to give this blue color when similarly treated, but stain a dull yellow passing to reddish under conditions favoring gradual concentration of the test solution. It was found, moreover, that individuals heterozygous for the waxy character produce both types of pollen grains, namely, blue-staining and yellowish-staining, in approximately equal numbers. This significant fact we took to indicate an immediate effect of the segregation of the non-waxy–waxy allelomorphs.

KIESSELBACH and PETERSEN have been unable to demonstrate this difference in the nature of waxy and non-waxy pollen and hold that it does not exist. Their observations and conclusions may be briefly summarized as follows: (1) Normally-developed mature pollen from waxy as well as from non-waxy races of maize gives the starch reaction with iodine. (2) The iodine solution does not serve to differentiate the content of the pollen grains of waxy and non-waxy maize. (3) The walls of the immature pollen of all varieties assume a reddish color with iodine which

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in older material is obscured by the dark stain of the starch within the grain. (4) Starch deposition in the grains of a single anther is sometimes not completed simultaneously, resulting in a transition stage in which some of the pollen stains reddish and some blue. (5) This latter condition was mistaken by the present investigators as evidence of segregation of the non-waxy–waxy genes.

It was made clear, we thought, at the time our discovery was reported, that the difference in the characters of the male gametophytes occasioned by the non-waxy-waxy allelomorphs rests upon a distinctive color reaction with iodine, of the two types of pollen *reserves*. It was pointed out that such difficulties in observation as were occasioned by the pollen-grain wall may be readily overcome by resort to the simple expedient of applying enough pressure to the cover-glass of the mount to rupture the grains and force out a portion of their contents. If attention is then focussed upon the granular inclusions of the cytoplasm, a difference in these materials from non-waxy and waxy gametophytes is readily apparent. In the case of pollen from homozygous non-waxy plants numerous small, mostly rod-shaped and ovate, blue-staining starch grains are present; the corresponding bodies from waxy pollen, which are similar in size but somewhat more irregular in shape, fail to give this blue reaction with iodine. If the preparation is allowed to stand unsealed for an hour or so, a little fresh solution being added from time to time, these waxy-type granules assume a reddish color. Plants segregating for waxy produce these two sorts of pollen with equal frequency.

Since this phenomenon was first reported, one of us (BRINK) has confirmed the difference in the nature of the reserve carbohydrates in non-waxy and waxy pollen by separating these substances in relatively pure form and studying their color reactions *in vitro*. Freed from both the spore coat and the cytoplasm in which they are found, it can be demonstrated in an unequivocal manner that the two types of reserves, when stained with iodine, provide a reliable basis for distinguishing non-waxy and waxy pollen. Although they do not stain as readily, these constituents of the pollen can be differentiated quite as clearly as the corresponding endosperm reserves. If small amounts of the granular carbohydrate reserves separated from the pollen of non-waxy and waxy plants are heated to boiling with water and a few drops of iodine added to the suspensions after cooling, the color reactions obtained are practically identical with those given by the corresponding materials from the endosperm. Following RIDGWAY'S (1912) standards, the color of the non-waxy preparation is Helvetia Blue, while that of the waxy preparation is Rood's Violet.

We cannot give credence to the statement by KIESSELBACH and PETERSEN that mature pollen of waxy and non-waxy races alike gives the typical starch reaction with iodine, nor can we let their observation that no difference is shown in the color reaction of the contents of the two sorts of crushed grains pass unchallenged. Of course, one may stain both types of pollen so heavily that they will appear black simply through failure to transmit light. In getting a satisfactory differentiation some discrimination as to strength of solution and time of staining must be exercised, to be sure, but the conditions of a satisfactory test are not difficult of fulfillment.

The argument that the difference which we have taken to indicate the effect of segregation, is a transitory condition, not only fails to account for the 1 : 1 distribution of the two types of pollen, but is not founded on fact. We have samples of pollen, collected at the time of dehiscence from non-waxy and waxy plants, which have been stored in the laboratory over calcium chloride for some eight months, which are as readily distinguished from each other now as when they were fresh.

As proved by the breeding results we have found the iodine reaction at pollinating time a uniformly dependable means of separating homozygous non-waxy from heterozygous plants in progenies comprising these genotypes. There is little ground for questioning the validity of a test which satisfies this criterion.

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THE MULTIPLE STOCK "XPLE" AND ITS USE

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Both in the construction and in the use of a map that shows the relative location of the genes contained in a given chromosome, it is necessary to have a table of corrections which gives for each two loci the amount by which the number of units of map-distance is greater than the percentage number of recombinations that is directly observed in an experiment. A few examples will make this clear. The percentage of recombinations for two linked characters is usually found by a "backcross" experiment, in which a female heterozygous for the two pairs of characters is mated to a male homozygous for the recessives of both pairs. Thus, if the recessive scute (lack of certain bristles from the scutellum) is crossed to the recessive echinus (a larger eye with coarser hairs on it) the F_1 female will be wild-type, for each parent contributes the recessive of one pair and the dominant wild-type allelomorph of the other pair $\left(\frac{s_e \quad +^{e_e}}{+^{s_e} \quad e_e}\right)$. This F_1 female is then mated to a male homozygous for both scute and echinus. The offspring are found to be of four classes. Two, scute and echinus, respectively, represent the original combinations of the four characters dealt with (scute with not-echinus, and not-scute with echinus). Two other classes are scute echinus and wild-type. These represent new combinations of the two pairs of characters that entered the cross (scute with echinus and not-scute with not-echinus). The percentage of recombination is found by dividing the sum of the two recombination classes by the total number in all classes, and multiplying by a hundred. A representative scute echinus backcross experiment (data from table 5) gave: $s_e = 9805$, $e_e = 9575$, $s_e\ e_e = 686$, $+ = 720$. The total recombinations were 1406, which is 6.8 percent of the total of 20,786 flies. With the crossveinless wing-character, scute gave 16.3 percent of recombination. In a given linkage group each two pairs of characters have a characteristic recombination percent.

The recombinations of linked characters is explained by "crossing over" of the chromosomes. In the cells that are about to give rise to eggs, the chromosome carrying scute comes to lie closely side-by-side with the homologous chromosome carrying vermilion. At some point or points

along the synapsed chromosomes the two chromosomes break across, and then rejoin in such a way that the part of each that lies to the left of the break is united with the part of the other that lies to the right of the break. Such points of crossing over may occur anywhere along the chromosome, and whenever one falls between the loci occupied by scute and echinus it gives rise to crossover chromosomes, one of which carries both mutant genes and the other of which carries neither. The individuals that come from the crossover chromosomes show recombinations of characters. The further apart the loci of two characters are, the more frequently will crossovers fall between them, and the higher will be the percentage of recombinations.

But if the loci of two characters are far enough apart (which is true for scute and crossveinless) two crossovers sometimes fall between them. In such an event, the genes that were together originally remain together in spite of the double crossing over between them. A section of chromosome lying between two genes is replaced by a section from the other homologue. No recombination of the characters is produced, and for each such case the total number of crossovers is increased by two, while the total number of recombinations of the linked characters is not increased at all. Thus the number of recombinations corresponds to the full number of crossovers only in case the two loci considered are so close together that no double crossing over occurs between them. In constructing the maps it is necessary to know the full number of crossovers (doubles counting as two, triples as three crossovers) that occur between the loci that are to be mapped. A unit of map distance corresponds to a section of chromosome of such length that one crossover falls within it for each average hundred gametes. In constructing the map of the first chromosome which is given as the base line in figure 1, scute and echinus were placed 6.8 units apart, which means that in the data on which that map was based (table 5) 6.8 crossovers between scute and echinus occurred for each hundred gametes. None of these were doubles, since scute and echinus are too close together to allow double crossing over between them. Scute and forked are there mapped as 62.0 units apart. Scute and forked are so far apart that not only double, but triple and quadruple crossing over has been found to occur between them. In constructing such maps it is first necessary to determine experimentally the amounts by which the various crossing over values exceed the directly observed recombination percents.

The experimental determination of the amount by which crossing over exceeds recombination is made through use of loci lying between the two

loci in question, and which are so close together that no double crossing over occurs between succeeding ones. Thus, the number of double crossovers occurring between scute and crossveinless can be determined through use of the intermediate locus echinus, the scute-echinus interval and the echinus-crossveinless interval being free of double crossing over. An F_1 female from the cross between scute crossveinless and echinus $\left(\frac{s_c \; +^{e_c} \; c_v}{+^{s_c} \; e_c \; +^{c_v}}\right)$ is backcrossed to a scute echinus crossveinless male. All double crossovers that occur between scute and crossveinless are easily detectable, since they result in $s_c \; e_c \; c_v$ and in wild-type flies, the number of which can be counted. In the data of table 5 there were 0.03 percent such doubles, which gives a difference of 0.06 between crossing over and recombination. This difference is very slight, and it is partly because it is so slight (and partly from direct tests) that it is certain that no doubles occur within either of the two smaller sections. To determine the difference for loci as far apart as scute and forked requires the simultaneous use of several intermediate loci.

The selection of these intermediate loci is a problem of no little difficulty, and it is only recently that mutant loci of suitable characteristics have become available. First, only mutant characters that are separable from the wild-type without error may be used. This requires a character that does not fluctuate greatly and for which the gap between the wild-type and the fluctuant closest to the wild-type is sharp. Second, the mutants must have practically normal viability, fertility and productivity. Some mutants that themselves have normal viability show great mortality if they are combined with certain other mutants. Thus, sable has been found to greatly decrease the viability of multiple stocks. Third, those used together must be sharply separable from one another, and every class possible from combination of the constituent characters must be sharply separable from every other class. Thus, ruby and garnet eye colors cannot be used together, nor singed and forked bristles. From the suitable mutants as small a number should be selected as possible and still have the number of doubles between successive loci either zero, or negligibly small (less than one double per 2000 flies), or small and of an amount known from other experiments.

In 1916 the best selection for the scute to forked interval was echinus, cut, vermilion and garnet. To carry out backcrosses a multiple stock containing these recessives together with scute and forked was necessary. The $s_c \; e_c \; c_t \; v \; g \; f$ multiple recessive was made by BRIDGES and called "Xple" (X-chromosome multiple). The Xple stock was used in the COLUMBIA

laboratory in finding the locus of several sex-linked mutants, and was sent to various workers elsewhere, who used it in studying the effect upon crossing over of several environmental and genetic agencies. Thus, PLOUGH (1921) used Xple in experiments that confirmed the finding of BRIDGES (1915) that the age of the mother made little or no difference in the crossing over frequencies for the X chromosome. By use of the same stock PLOUGH showed that heat treatment was likewise without any certain effect. GOWEN and GOWEN (1923) used Xple to show that a third-chromosome recessive gene suppressed all crossing over in the X chromosome. MOHR (1923b) used Xple in testing the length of the deficient section due to notch 8, and in showing that crossing over outside of the deficient section itself is not affected. Mrs. WARD (1923) used Xple to test the effect upon crossing over in the X chromosome of curly, which suppressses nearly all crossing over in the second chromosome. The data indicated that curly influences crossing over in the X to a slight extent. Mohr (1923a) used Xple in finding the locus of a semilethal character chlorotic, whose locus is slightly to the left of scute.

In table 1 is given a summary of the data involving Xple. In table 2 are given the data secured by MOHR in the cross of notch 8 by Xple. The

TABLE 1

Summary of data involving $\frac{s_o\ (1)\ e_c\ (2)\ c\ \ (3)\ v\ (4)\ g\ (5) f}{+\ \ +\ \ +\ \ +\ \ +\ \ +}$.

SOURCE OF DATA	CROSSOVER REGIONS										
	0	1	2	3	4	5	1,2	1,3	1,4	1,5	2,3
PLOUGH '21	2,216	204	575	521	393	424	17	44	56	60	25
MOHR '23b; ♂♂	434	56	131	135	57	55	..	8	6	4	4
MOHR '23b; ♀♀*	659	73	193	175	105	111	..	5	11	7	5
Total	3,309	333	899	831	555	590	17	57	73	71	34

TABLE 1 (continued)

SOURCE OF DATA	CROSSOVER REGIONS											
	2,4	2,5	3,4	3,5	4,5	1,2,3	1,2,5	1,3,5	1,4,5	2,3,5	2,4,5	3,4,5
PLOUGH '21	63	77	13	39	10	1	1	1	1	1	1	3
MOHR '23b; ♂♂	7	16	1	7	1	..	..	..	..	..	..	..
MOHR '23b; ♀♀*	8	22	3	9	..	..	..	..	..	..	..	..
Total	78	115	17	55	11	1	1	1	1	1	1	3

* Data unpublished, kindly supplied by MOHR.

TABLE 2

Data from MOHR (1923 a) *involving* $\frac{s_c + e_c\ (2)\ c_t\ (3)\ v\ (4)\ g\ (5)\ f}{+ N_8 + \quad + t \quad + \quad + \quad +}$.

CROSSOVER REGIONS												
0	2	3	4	5	2,3	2,4	2,5	3,4	3,5	4,5	2,3,4	2,3,5
897	213	251	162	155	4	25	36	11	28	7	1	2

data of table 2 and the other data involving notch 8 show that the crossing-over relations to the right of echinus are probably not affected by the action of notch 8, and accordingly these data can be used in connection with those of table 1. The combined data give the following percentagesof recombination for the successive sections: s_c-$e_c = 7.9$, e_c-$c_t = 16.4$, c_t-$v = 14.6$, v-$g = 10.6$, g-$f = 12.0$. The map based on these data would be about 62.0 units long from scute to forked, which is slightly longer than the old standard map of 56.5 (BRIDGES 1921). The greatest difference is in section 1 (7.9 instead of 5.5) and in section 2 (16.4 instead of 14.5).

On the basis of the data of table 1 alone the scute-forked map distance is 60.9, while the scute-forked recombination percentage is 45.7. That is, each hundred flies involved 15.2 cases of crossing over that did not give rise to recombinations for scute and forked. Discussion of the other points brought out by these data will be deferred until further data have been presented.

In 1919 the excellent sex-linked recessive mutant "crossveinless" was discovered (BRIDGES 1920), and its locus was found to be about half way between those for echinus and cut. By use of crossveinless the distance of about 15 units between echinus and cut could be broken into two sections of about 8 and 7 units. Each of these sections would be so short that no double crossing over would occur within it, and the number of the doubles occurring between echinus and cut could be accurately determined by direct experiment. An X-multiple that includes crossveinless in addition to the others already in use would be much more effective. Also, two other mutants had been discovered by BRIDGES that offered improvements over those in the old Xple. The mutant cut in the old Xple was the original cut found by BRIDGES in 1915. An allelomorph of cut, namely cut^6, was found that was more viable than the original cut and that did not affect the eyes, antennae, and abdomen to such an extent as the other allelomorphs. Classification was perfect on the basis of the wing character, the wing being "cut" to a sharp point. Likewise a new allelomorph of garnet, namely, garnet^2, was found, that had a lighter eye

TABLE 3

Backcross data $\frac{s_c\ (1)\ e_c\ (2)\ c_v\ (3)\ c^6\ (4)\ v\ (5)\ g^2\ (6)\ f}{+\ +\ +\ +\ +\ +\ +}$.

CULTURE NUMBER	CROSSOVERS IN REGION																											
	0		1		2		3		4		5		6		1,2		1,3		1,4		1,5		1,6		2,3	
	s_c e_c c_v c_t v g f	+	s_c c_v c_t v g f	e_c	s_c e_c c_v v g f	c_t	s_c e_c c_v c_t v g f	v	s_c e_c c_v c_t v g f	v	s_c e_c c_v c_t v g f	g	s_c e_c c_v c_t v g f	f	s_c c_v c_t v g f	e_c	s_c c_t v g f	e_c c_v	s_c v g f	e_c c_v c_t	s_c g f	e_c c_v c_t v	s_c e_c c_t v g f	c_v	s_c e_c v g f	c_v c_t
2	46	55	6	6	7	7	9	15	16	19	12	15	5	10	..	1	..	..	1	1	..	2	1	1	..	..
4	65	58	16	3	11	9	9	9	16	13	12	6	11	9	..	..	..	..	..	2	2	..	..	.	..	..
6	42	54	16	7	10	9	12	6	17	14	13	13	9	8	..	..	1	1	2	3	..	5	1	1	..	..
7	73	87	11	7	24	12	15	13	21	18	12	10	9	13	..	..	..	..	2	..	..	..	..	1	1	..
9	71	109	21	13	23	18	20	26	26	34	19	21	9	13	..	..	..	..	1	..	4	..	4	5	..	..
11	28	92	17	6	15	14	13	13	17	28	19	19	4	16	..	..	..	..	..	2	..	4	1	1	..	..
12	86	133	23	13	20	22	19	18	37	25	14	17	13	17	..	..	1	..	..	2	1	1	..	2	..	..
13	61	78	9	9	15	4	16	7	24	23	11	19	15	15	..	..	..	..	2	4	3	..	..	1	..	..
14	19	29	7	4	5	7	6	3	2	10	7	12	5	8	..	..	..	..	..	..	1	..	1	1	..	..
16	70	104	11	14	10	11	11	16	23	25	12	14	14	17	1	..	..	..	1	2	2	3	1	2	..	..
Totals	561	799	127	82	140	113	130	126	199	209	121	146	94	126	1	1	2	1	9	16	13	15	9	15	1	0
	1360		209		253		256		408		267		220		2		3		25		28		24		1	

TABLE 3 (continued)

CULTURE NUMBER	CROSSOVERS IN REGION																							
	2,4		2,5		2,6		3,4		3,5		3,6		4,5		4,6		5,6		1,4,5		1,4,6		2,4,6		3,5,6	
	S_e e_e v g f	c_v c_t	S_e e_e g f	c_v c_t v	S_e e_e f	c_v c_t v g	S_e e_e c_v v g f	c_t	S_e e_e c_v c_t g f	v	S_e e_e c_v c_t v g f		S_e e_e c_v c_t g f	v	S_e e_e c_v c_t v g f		S_e e_e c_v c_t v g f		S_e e_e c_v c_t v g f		S_e e_e c_v c_t v g f		S_e e_e c_v c_t v g f		S_e e_e c_v c_t g f	v
2	2 ..		2 1		2 1		1 1		.. ..		1 ..		2 2		1 2		.. ..		.. ..		.. ..		.. ..		.. ..	
4	.. 1		1 1		4 1		.. ..		.. ..		.. 1		3 ..		2 1		.. ..		.. ..		.. ..		.. ..		.. ..	
6	2 ..		2 1		1 3		1 ..		.. 1		.. ..		1 ..		2		1 ..		.. ..		.. ..		.. ..		1 ..	
7	1 1		3 1		5 1		.. ..		.. ..		.. 1		1 1		5 1		.. 1		.. ..		.. ..		.. ..		.. ..	
9	.. 2		2 2		3 1		1 ..		.. 1		.. ..		.. ..		1 3		1 ..		1 ..		2 ..		.. ..		.. ..	
11	1 ..		1 2		.. 2		.. 1		1 1		2 ..		1 ..		2 2		.. ..		.. ..		.. 1		.. ..		.. ..	
12	.. 3		2 4		4 1		.. 1		3 2		3 1		.. 2		4 5		.. ..		.. ..		.. ..		.. ..		.. ..	
13	2 2		2 ..		3 ..		.. 1		2 1		5 1		.. ..		3 ..		1 ..		.. ..		.. ..		.. ..		.. ..	
14	.. ..		.. ..		2 ..		.. ..		.. ..		1 2		.. 1		1 3		.. 1		.. ..		.. ..		.. ..		.. ..	
16	1 ..		1 1		1 3		.. ..		.. ..		.. 2		.. 2		.. 1		1 1		.. ..		.. ..		1 ..		.. ..	
Totals	9 9	18	16 13	29	25 13	38	3 4	7	6 6	12	12 8	20	8 8	16	19 20	39	4 3	7	1 0	1	2 1	3	1 0	1	1 0	1

color than the original garnet and that could be separated from the wild-type with greater speed and sureness. A second advantage of garnet^2 over garnet is that the vermilion garnet^2 double recessive is a light yellow-orange color easily separated from garnet^2, while the vermilion garnet double recessive is a darker red-orange that is not strikingly different, especially in older flies, from garnet itself. Accordingly, a new Xple was constructed in 1920 with the formula $s_c\ e_c\ c_v\ c_t{}^6\ v\ g^2 f$. This Xple has replaced the old both at the COLUMBIA laboratory and at the other laboratories where the most active work is being carried on.

The new Xple had come from various sources, and included only parts of the old Xple. There had been opportunity for a new set of modifiers of the amount of crossing over to be brought together. It was not anticipated that any of these modifiers would be of striking effect, for the stocks used were known to give at least approximately standard values. To test the condition of the new Xple a backcross was made by OLBRYCHT, whose results are given in table 3.

According to the data of table 3 the recombination percentages for the various sections of the Xple were: s_c-e_c=9.0; e_c-c_v=10.5; c_v-c_t=9.2; c_t-v=15.9; v-g=11.2; g-f=10.9. The length of the map from scute to forked is 66.7 units, which is 10.2 units longer than the standard map. Again the increase was largest at the extreme left end and gradually decreased toward the right until the g-f value was slightly below the old standard. The s_c-f recombination percent was 49.8, which is 16.9 lower than the crossover value.

An examination of the details of table 3 shows a striking characteristic of the pairs of complementary classes that constitute the crossover classes. In a given crossover class the two complementary classes are expected to be equal, since the two classes of gametes from which they come are equal. But if the zygotes of one class have a heavier mortality than the zygotes of the complementary class their inequality appears in the ratio of zygotes. In crossover classes 3 and 4 the complementary classes are very nearly equal (130 : 126 and 199 : 209), but in crossover classes 1 and 6 they are quite unequal (127 : 82 and 94 : 126). In crossover class 1 the heavy mortality of the $e_c\ c_v\ c_t{}^6\ v\ g^2 f$ class is due to the summation and interaction of six mutant characters, while the relatively slight mortality of the complementary class is due to the single mutant s_c. In crossover class 4 the mortality of the combination of four mutants $s_c\ e_c\ c_v\ c_t{}^6$ is only slightly greater than that of three mutants $v\ g^2 f$; and the same is true in class 3 for $c_t{}^6\ v\ g^2 f$ and $s_c\ e_c\ c_v$. In crossover classes 2 and 5 the complementary classes have 2 and 5 mutant characters, and the inequality

is intermediate between that due to a 3-4 division and to a 1-6 division. It is to be expected that the 7-0 division, namely, Xple and +, as it exists in the non-crossover class, would give the greatest departure from equality of complementary classes. This is undoubtedly the case, but the above data have been selected in such a way as to exclude cultures in which the inequality of Xple and wild-type was strikingly great. The selection was made in the following manner: About 15 cultures were started originally and after they had been hatching a few days the five cultures in which the inequality between Xple and wild-type was greatest were discarded and the counts were completed only for the 10 cultures in which the classes seemed fairly equal. The justification for this selection lies in the fact that those cultures in which the greatest inequalities exist are those in which the culture conditions are most adverse to normal viability, and in which the disturbing effect of differential viability upon the various crossover classes is greatest. The best single index of the normality of viability conditions is the Xple to wild-type ratio, because the greatest effect is expected there, and because in the above experiment those classes were the non-crossover classes and hence the largest classes present. The correlations between the mortality disturbances in the various classes is large, but not so large that a selection based primarily on the Xple and wild-type classes would give equally normal ratios in the other classes. Hence, in the above data the ratio in the 7-0 division (41.2 : 58.8) departs from equality more than one of the 6-1 divisions ($s_c\ e_e\ c_t^6\ v\ g^2 : f = 42.7 : 57.3$), but not more than the other ($e_e\ c_t^6\ v\ g^2 f : s_c =$ 39.2 : 60.8). The difference is slight, and for the primary purpose of the experiment, the determination of recombination percentages and crossover values, the data are more valid than they would be if no selection had been made.

TABLE 4

Ratios of complementary classes from table 3.

DIVISIONS	COMPLEMENTARY CLASSES	RATIO
7–0	$s_e\ e_e\ c_v\ c_t\ v\ g\ f$: wild-type	41.2 : 58.8
6–1	$s_e\ e_e\ c_v\ c_t\ v\ g$: f	42.7 : 57.3
5–2	$s_e\ e_e\ c_v\ c_t\ v$: $g\ f$	45.3 : 54.7
4–3	$s_e\ e_e\ c_v\ c_t$: $v\ g\ f$	48.8 : 51.2
3–4	$s_e\ e_e\ c_v$: $c_t\ v\ g\ f$	50.8 : 49.2
2–5	$s_e\ e_e$: $c_v\ c_t\ v\ g\ f$	55.3 : 44.7
1–6	s_e : $e_e\ c_v\ c_t\ v\ g\ f$	60.8 : 39.2
0–7	Wild-type: $s_e\ e_e\ c_v\ c_t\ v\ g\ f$	58.8 : 41.2

TABLE 5

Alternated backcross of Xple $\frac{s_s \quad c_v \quad v \quad f}{(1)\ e_c\ (2) \quad (3)\ c^{+}\ (4) \quad (5)\ g^{+}\ (6)}$.

CULTURE NUMBERS	0		1		2		3		4		5		6		1,2		1,3		1,4	
	s_t c_v v f	e_c c_t g	s_s e_c c_t g	c_v v f	s_s c_t g	e_c c_v v f	s_s c_v c_t g	e_c v f	s_s c_v g	e_c c_t v f	s_s c_v v g	e_c c_t f	s_s e_c c_v v	c_t g f	s_s e_c c_v v f	c_t g	s_s e_c v f	c_v c_t g	s_s c_c c_t v f	c_v g
1	61	57	6	5	6	11	5	9	10	13	14	9	11	13	..	..	1	..	1	..
2	81	77	3	2	16	13	14	11	26	22	16	16	7	11	..	..	..	..	..	..
3	128	101	13	15	16	15	22	23	27	31	32	23	16	29	..	..	..	..	..	1
4	110	106	4	8	11	14	10	9	25	15	18	16	21	8	..	..	..	..	..	..
5	122	127	12	12	24	12	28	15	32	25	29	21	24	19	..	..	..	..	1	2
6	69	103	8	9	12	24	7	16	19	19	29	12	19	17	..	..	..	..	..	..
7	115	107	13	15	16	19	12	19	31	24	9	16	16	6	..	..	..	..	2	..
8	58	56	4	6	7	9	15	6	12	22	12	18	10	8	..	..	..	..	..	..
9	78	77	8	12	12	12	20	13	20	22	26	24	28	16	..	..	..	..	..	1
10	77	69	4	9	11	8	10	3	22	20	17	12	11	13	..	..	..	2	1	2
11	75	61	8	3	11	12	9	10	24	17	9	14	10	9	..	..	..	..	1	1
13	85	90	11	14	18	21	8	15	29	20	16	16	17	10	..	..	..	..	1	..
15	86	76	9	6	18	10	16	10	21	22	21	11	14	9	..	..	..	..	1	..
16	52	80	16	14	7	8	4	8	19	15	12	13	13	21	..	..	..	..	1	..
Totals	1197	1187	119	130	185	188	179	167	317	287	251	221	217	189	..	..	1	2	9	7
(A) xx	980	922	97	94	144	134	112	160	239	237	140	175	169	158	1	..	1	..	6	9
(B) xxy	767	748	61	66	117	114	101	107	177	205	135	130	114	119	1	..	..	3	5	5
(C) xx	421	443	49	65	73	69	69	60	129	119	82	88	92	70	..	..	..	..	2	5
(D) xxy	888	808	74	105	108	152	139	149	240	227	142	135	102	120	1	..	1	1	9	9
(E) xxy	635	558	72	62	96	85	85	97	157	178	113	105	101	74	..	..	1	..	15	6
Grand total	4888	4666	472	522	723	742	685	740	1259	1253	863	854	795	730	3	0	4	6	46	41
	9554		994		1465		1425		2512		1717		1525		3		10		87	

TABLE 5 (continued)

CULTURE NUMBERS	1,5		1,6		2,3		2,4		2,5		2,6		3,4		3,5		3,6		4,5		4,6	
	Sc *ec* *Ct* *f*	*Cv* *v* *g*	*Sc* *ec* *Ct* *g* *f*	*Cv* *v*	*Sc* *v* *f*	*ec* *Cv* *Ct* *g*	*Sc* *Ct* *v* *f*	*ec* *Cv* *v* *g*	*Sc* *Ct* *f*	*ec* *Cv* *v* *g*	*Sc* *Ct* *g* *f*	*ec* *Cv* *v*	*Sc* *Cv* *Ct* *f*	*ec* *v* *g*	*Sc* *Cv* *Ct* *v* *f*	*ec* *g*	*Sc* *Cv* *Ct* *g* *f*	*ec* *v*	*Sc* *Cv* *f*	*ec* *Ct* *v* *g*	*Sc* *Cv* *g* *f*	*ec* *Ct* *v*
1	1 1		2 ..		.. ..		.. 1		.. 1		1 1		.. ..		1 2		2 1		.. ..		2 ..	
2	1 ..		.. 1		1 ..		.. ..		1 1		2 3		.. ..		2 ..		2 1		4 ..		3 5	
3	.. 1		1 1		1 ..		.. 2		2 4		2 2		.. ..		1 3		3 3		1 ..		7 3	
4	2 ..		3 2		.. ..		2 2		3 5		1 5		.. ..		1 ..		1 1		1 1		3 4	
5	3 4		1 ..		1 ..		3 ..		3 4		4 4		.. 1		2 2		3 6		2 2		2 3	
6	.. 1		3 ..		.. ..		2 3		3 3		1 ..		.. ..		1 ..		.. 6		4 1		3 4	
7	1 1		4 ..		.. ..		5 2		6 1		6 3		.. 1		.. 3		3 5		.. 1		2 2	
8	1 1		1 1		.. ..		.. 1		1 ..		2 ..		.. ..		.. ..		.. ..		1 ..		1 2	
9	1 4		1 1		.. ..		1 ..		1 1		4 2		.. ..		1 2		1 3		.. 4		5 5	
10	1 ..		1 ..		1 ..		.. 2		1 5		2 2		.. ..		2 ..		2 2		.. ..		2 4	
11	1 ..		2 3		.. ..		.. ..		5 ..		3 3		.. ..		2 ..		1 ..		.. 1		2 4	
13	3 ..		2 ..		.. ..		.. ..		1 2		1 5		1 ..		2 2		3 1		.. 1		2 4	
15	.. 1		3 3		.. ..		1 ..		3 3		.. 5		.. ..		2 1		.. ..		2 ..		1 ..	
16	.. 1		1 1		.. ..		1 1		1 1		5 1		.. ..		.. ..		1 1		.. 1		2 2	
Totals	15 15		25 13		4 ..		15 14		31 31		34 36		2 2		17 15		22 30		15 13		37 42	
(A)xx	12 12		13 18		1 ..		4 5		15 19		24 18		3 2		7 10		15 12		5 8		19 16	
(B)xxy	13 8		11 6		1 ..		11 6		11 10		14 17		1 2		12 10		13 10		4 5		20 12	
(C)xx	4 9		7 8		.. ..		7 4		8 7		15 8		1 2		6 3		5 10		6 2		14 10	
(D)xxy	13 18		12 10		.. 2		5 8		15 21		14 21		4 4		12 10		11 14		6 8		15 15	
(E)xxy	12 9		10 14		.. ..		10 6		15 8		17 12		1 2		5 3		4 15		1 3		12 13	
Grand total	69 71	140	78 69	147	6 2	8	52 43	95	95 96	191	118 112	230	12 14	26	59 51	110	70 91	161	37 39	76	117 108	225

TABLE 5 (continued)

CULTURE NUMBERS	5,6	1,2,6	1,3,5	1,3,6	1,4,5	1,4,6	1,5,6	2,3,4	2,4,5	2,4,6	2,5,6	3,4,6	3,5,6	4,5,6	1,2,3,4	1,2,3,6
	s_e e_e c_v cl v g f	s_e e_e c_v v	s_e e_e v g	e_e c_v cl g f	s_e cl v g c_v f	s_e e_e cl v c_v g f	c_v v g f	s_e g	e_e c_v f	s_e e_e cl v c_v g f	e_e c_v v g f	s_e e_e c_v cl v g f	s_e e_e c_v cl cl v g f	s_e c_v g	s_e e_e c_v cl g f
0	.. ..	..	..	..	.. ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
2	.. ..	..	..	..	.. ..	.. ..	..	..	..	.. ..	..	.. ..	1 ..	..	..	..
3	2 ..	..	..	..	.. ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
4	1 3	1	..	..	1 ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
5	.. 3	..	..	..	1 ..	.. ..	..	..	..	.. 1	..	.. ..	.. ..	..	1	1
6	.. 1	..	..	..	.. ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
7	.. 1	..	..	..	.. ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	1	..	..
8	.. 1	..	..	..	.. ..	.. ..	..	..	1	.. 1	..	.. ..	.. ..	..	..	..
9	.. ..	..	..	..	.. ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
10	1 ..	..	..	..	.. ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
11	1 1	..	1	..	1 ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
13	.. 1	..	..	1	1 ..	1 ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
15	2 1	..	..	1	.. ..	.. ..	..	..	..	.. ..	..	.. ..	.. ..	..	..	..
16	.. ..	..	..	..	.. ..	1 ..	..	..	..	.. ..	..	.. 1	.. ..	..	..	..
Totals	7 12	1	1	2	4 ..	2 ..	..	..	1	.. 2	..	.. 1	1 ..	1	1	1
(A)xx	3 5	..	..	..	.. ..	1 1	..	..	..	.. ..	..	.. 1	.. ..	..	..	..
(B)xxy	1 2	..	..	..	.. ..	.. 1	..	1	..	.. ..	..	.. ..	.. ..	..	..	..
(C)xx	3 2	..	..	..	.. ..	.. ..	1	..	..	.. ..	1	.. 1	.. ..	..	..	..
(D)xxy	4 4	..	1	1	1 2	1 2	..	..	..	2 ..	..	.. ..	.. 1	..	..	..
(E)xxy	2 1	..	..	..	.. ..	1 ..	..	..	..	1 ..	..	1 ..	.. ..	..	..	..
Grand total	20 26	1	2	3	5 2	5 4	1	1	1	2 2	1	1 3	1 1	1	1	
	46	1	2	3	7	9	1	1	1	4	1	4	2	1	1	1

With the exception just noted, there is a regular seriation in the ratios of complementary classes in the above data (see table 4). It is further to be noted that the number of characters has far greater weight than the particular kind. This is due to the fact that in making Xple only such characters were included as were known to have excellent viability.

The inviability effects will be equalized and neutralized to the greatest extent if the number of mutants in complementary classes is equalized as much as possible. This is accomplished by the "alternated backcross" (BRIDGES 1919, 1921), in which half the characters enter from one parent and half from the other, and in which characters consecutive in the map alternate in the homologous chromosomes of the heterozygous female. Thus, the seven characters of the Xple would be divided into 4 and 3 and the parental combinations would be $s_c\ c_v\ v\ f$ and $e_c\ c_t{}^6\ g^2$. The heterozygous female would be $\frac{s_c\ +^{s_c}\ c_v\ +^{c}\ v\ +^{g^2}\ f}{+^{s_c}\ e_c\ +^{c_v}\ c_t{}^6\ +^{v}\ g^2\ +^{f}}$, or more simply formulated, $\frac{s_c\ \ c_v\ \ v\ \ f}{(1)\ e_c\ (2)\ (3)\ c_t{}^6\ (4)\ (5)\ g^2\ (6)}$. The non-crossover complementary classes contain 4 and 3 mutants, and so also do each of the pairs of complementary classes due to crossing over in the six crossover sections. Also, a majority of the pairs of complementary classes due to multiple crossing over are divisions into 4 and 3. (Thus, 1, 3 double crossing over gives the two classes $s_c\ e_c\ v\ f$ and $e_c\ c_t{}^6\ g^2$). Only a small proportion of the flies would be in 2-5 divisions, while almost no flies would be in 1-6 or 0-7 divisions.

The stocks of $s_c\ c_v\ v\ f$ and $e_c\ c_t{}^6\ g^2$ were prepared and crossed by BRIDGES and the F_1 wild-type females were backcrossed to Xple males. About 5000 flies of the alternated backcross were raised by OLBRYCHT (table 5, upper section) for direct comparison with the non-alternated results of table 3, and BRIDGES raised some 16,000 others in the course of studying the effect of the Y chromosome on crossing over in the XXY female (table 5, A, B, C, D and E).

In the total of 20,786 flies none was in the 7-0 division, and only 2, or 0.01 percent, were in 6-1 divisions. In the classes in which the division was 5-2, the total flies were 480, or only 2.2 percent. The ratio was 235 : 245, which is practically equality. All of the remaining flies, 20,304 or 97.8 percent, were in 4-3 divisions, and the ratio in the totals was 10,205 : 10,099, which is very close to equality.

In the non-crossover class and each of the single-crossover classes the number of flies was large, and in each case the complementary classes

were practically equal. It is thus seen that the alternated-backcross method was successful in distributing the mortality uniformly throughout the population.

The recombination percents for the various sections are s_c-e_c=6.8, e_c-c_v=9.6, c_v-c_t=8.4, c_t-v=14.7, v-g=11.0, g-f=11.4. Only one of these sections, namely, c_t-v, is long enough for double crossing over to occur within it. Experiments in which tan or lozenge was present between cut and vermilion have shown that there is roughly one double crossover between cut and vermilion for each 2000 flies. The map value for c_t-v is therefore 0.1 larger than the observed recombinations percent (14.7 +0.1=14.8). The maps of the X chromosome corresponding to the alter-

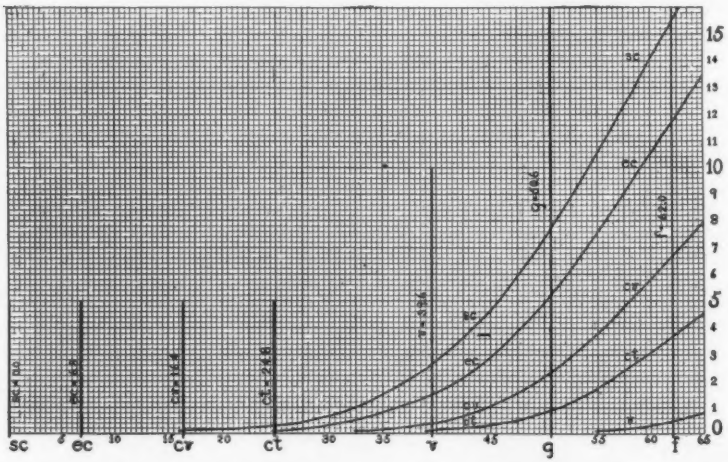

FIGURE 1.—Curves showing difference between map-distance and percentage of recombination for the characters of the alternated-Xple backcross.

nated Xple is given as the base line of figure 1. In comparison with the standard map (BRIDGES 1921) the alternated-Xple map is longer in the sections to the left of vermilion and shorter to the right of garnet. It is important to have a map based solely on alternated-Xple data, since the probability is that for several years the use of alternated Xple will be the standard method for testing crossing over in the X chromosome.

The crossover value for scute and forked on the basis of the data of table 5, is 62.0 units. With respect to scute and forked each average hundred gametes was found to consist of 45.91 non-crossovers, 46.37 single crossovers, 7.53 doubles, 0.18 triples and 0.01 quadruples. The total number of s_c-f crossovers is thus 46.37+2×7.53+3×0.18+4×0.01,

which totals 62.01. The percentage of recombinations for scute and forked was 46.55 (the sum of the singles and triples). The number of crossovers per hundred gametes thus exceeded the number of recombinations by 15.46. This difference is also directly obtained as $2\times7.53+2\times0.18+4\times.01=15.46$.

In figure 1 this difference of 15.5 is represented by the height of an ordinate at f. Likewise the s_c-g map distance has been calculated and found to be 50.6, and the corresponding recombination percent is 42.9, the difference of 7.7 being represented by the height of an ordinate at g. For s_c-v the difference is 2.6; for s_c-c_t it is 0.3; for s_c c_v it is 0.1. The smooth curve drawn through these experimentally determined points gives a means of predicting the difference between crossing over and recombinations for scute and any other locus. Thus, to find the expectation for the difference for scute and miniature, the locus of miniature is intercalated in the alternated-Xple map between vermilion and garnet with the same ratio between the v-m and m-g intervals as exists in the standard map, (3.1 : 8.3 : : 3.0 : 7.0). The proportionate locus of miniature is thus 3.0 units to the right of vermilion, or at 42.6, and the ordinate at 42.6 cuts the curve from scute at a height of 3.3 units. Similar smooth curves can be drawn through the other points determined by the above experiment, and predictions can thus be made for echinus and any other locus, for crossveinless and any other locus and for cut, vermilion and garnet and other loci.

Curves originating at other points can be intercalated between the curves originating at the points of Xple. Thus, a curve originating at ruby can be intercalated between those originating at echinus and at crossveinless, with the distance from the intercalated curve to the other two curves proportional to the e_c-r_b and the r_b-c_v map distances (2.0 : 6.2).

By extrapolation the curves can be continued beyond the locus of forked until they intersect the ordinates from fused and bobbed. There is also some direct evidence at hand as to the course of the curves beyond forked, so that the extensions need not be pure extrapolation.

The knowledge of the amount of corrections, gained principally from the above data, has been applied in the construction of a standard map with correction curves. This map covers the region to the right of forked, and has intercalated curves originating from the other principal loci besides those included in Xple. It has been published as part of the summary and review of the Drosophila work that appeared in Vol. II of Bibliographia Genetica under the editorship of LOTSY. A map with curves similar to that of figure 1, but based on smaller numbers in the

alternated backcross, has appeared in the CARNEGIE INSTITUTION OF WASHINGTON Year Book for 1924.

At present, experiments are under way to extend the information on the course of the curves in the region to the right of forked. These experiments make use of the dominant mutation minute-n, situated at about six units to the right of forked, and the recessive bobbed, situated about 13 units to the right of forked.

In constructing the old standard map (MORGAN and BRIDGES 1916) use was made of experiments that covered the length of the X chromosome with only a few intermediate loci, or that covered particular parts fairly closely. MULLER (1916) devised a method for covering the length of the X chromosome satisfactorily even with mutants that were poorly viable and hard to classify; but the method was so laborious, involving a genetic test of each individual recorded, that he secured only 712 individuals. For some unknown reason the amount of crossing over was abnormally low in MULLER'S data. Most of the later work suggests that a new standard map should be made, and that it would correspond rather closely to the alternated-Xple map presented here.

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THE SEGREGATION OF *OENOTHERA NANELLA-BREVISTYLIS* FROM CROSSES WITH *NANELLA* AND WITH *LAMARCKIANA*[1]

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INTRODUCTION

In earlier papers (DAVIS 1918, 1921) I have given a large body of data on the segregation of *Oenothera brevistylis* from crosses with *Oenothera Lamarckiana*. The results showed that the characters of *brevistylis* are associated and recessive to those of *Lamarckiana* and that the inheritance of *brevistylis* characters from the *Lamarckiana*-like F_1 reciprocal crosses, when selfed, presents in the F_2 a segregation of *brevistylis* and *Lamarckiana* plants in proportions close to the monohybrid ratio of 1 : 3. As would be expected, the same ratio was found in progeny from double reciprocals; that is, from crosses between the two F_1 generations. Finally, backcrosses of the F_1 plants to the recessive *brevistylis* gave segregation of *brevistylis* and *Lamarckiana* approximating the ratio 1 : 1. The results then, in support of earlier conclusions of DE VRIES, consistently showed that the characters of *brevistylis* are inherited as a group according to the simple Mendelian system for a monohybrid.

There were, however, rather constant variations from the expected ratios and always to the disadvantage of *brevistylis* plants, which appeared

[1] Genetical studies on Oenothera XIII. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 225.

in numbers not so large as expectations called for, and these smaller proportions seem correlated with a lower seed viability of *brevistylis*. These facts suggested that conditions which reduce the percentage of viable seeds would lower the proportions of *brevistylis* plants. The second paper (DAVIS 1921) gave the results of experiments which proved that malnutrition of developing seeds will kill a much larger proportion of *brevistylis* zygotes or embryos than those of *Lamarckiana*, and malnutrition may therefore be considered as at least one factor affecting the smooth expression of a simple Mendelian inheritance of the group of characters which distinguishes *brevistylis* from *Lamarckiana*.

In the cultures, which totalled about 5000 plants, the following well known "mutants" of DE VRIES were identified: 10 *albida*, 2 *elliptica*, 3 *laevifolia*, 3 *lata*, 12 *nanella*, 29 *oblonga*, 1 *rubrinervis*, and 16 *scintillans*. Of these, all but the *albida* and *elliptica* came to flower, and with one exception none of them presented the characters of *brevistylis*. The exception was a plant with the habit and stature of *nanella* (culture 20.76, DAVIS 1921, p. 580, table 3) in an F_2 from the cross *brevistylis* × *Lamarckiana*. The plant had the characters of *nanella* combined with all of the characters of the *brevistylis* complex, namely, short style with deformed or rudimentary stigma lobes, bud cones more stout and with short sepal tips, broader less-pointed bracts, and ovary partly superior and ripening no seeds. This plant, 20.76-34, was named *nanella-brevistylis*.

Nanella-brevistylis is a plant resulting from the association of *brevistylis* characters, which we shall term the short-style complex, with the dwarf stature and habit peculiar to *nanella*. It became then a matter of interest to determine what might happen when *nanella-brevistylis* was crossed to *nanella* and to *Lamarckiana*. From the cross with *Lamarckiana* information might come which would help to determine whether genes for stature and for style length were linked in the same chromosome or whether they lay in different chromosomes as suggested by SHULL (1921). Because the plant of *nanella-brevistylis* produced no seed when pollinated, the cross could be made only with it as the pollen parent.

All of the results reported in this paper are from cultures in which the seeds were forced to complete germination by the following method: After soaking for 24 hours the seeds were submitted, while still in water, to exhaust alternating with pressure of about 30 pounds, 6 to 8 times in a period of from 10 to 24 hours. The exhaust and pressure was effected in an iron case with suitable attachments to a pump operated by an electric motor. By this operation air was drawn out of the pores in the seed coats and water was forced into the interior of the seeds. Following this treat-

ment the seeds were placed on pads of wet filter-paper in Petri dishes and kept shaded at hothouse temperature. Most of the viable seeds sprouted within 4 to 6 days and germination was usually complete after two weeks. The germinating seeds were removed to other Petri dishes and a few days later their seedlings were set in pans. The residue of sterile seeds, after germination was believed to be complete, was tested by pinching each seed with a strong forceps to make sure that none contained embryos. After some experience it is not difficult to judge when germination in a Petri dish is finished; in the cultures here described no ungerminated seeds were found in the residue of sterile structures. The number of seedlings divided by the sum of sterile and fertile seeds gives the percentage of germination.

THE F_1 GENERATION OF *nanella*×*nanella-brevistylis*

The F_1 generation of *nanella* × *nanella-brevistylis* (culture 21.29) was through a plant of typical *nanella* (20.66-34) out of the F_2 of one of my crosses of *brevistylis*×*Lamarckiana*. This plant was pollinated from the original plant of *nanella-brevistylis* (20.76-34). The contents of one large capsule gave 144 seedlings of which 11 were stumpy,—that is, the seedling failed to develop the root from the end of the hypocotyl and soon died. There was a residue of 152 sterile seeds and, therefore, a germination of 48.6 percent. The seed fertility of my cultures of *nanella*, as tested in 1921, 1923 and 1924, was respectively 38.7, 44.3 and 39.6 percent. High mortality in the culture reduced its numbers to 98 plants which came to maturity. All of these were of the long-styled *nanella* type. Thus, the short-style complex of *nanella-brevistylis* is recessive to the long-style complex of *nanella*, as are the characters of *brevistylis* recessive to *Lamarckiana*. Two plants of this F_1 generation (21.29-1 and 21.29-2) were selfed in preparation for the F_2 generations.

THE F_2 GENERATIONS FROM *nanella*×*nanella-brevistylis*

Culture 23.49 was grown from the contents of 5 medium-sized capsules of the F_1 plant 21.29-1. Seedlings numbered 240, of which 49 were stumpy and of course died; 323 sterile seeds remained and the germination was 42.6 percent, which was close to the germination percentage of *nanella*. Mortality among the young plants reduced the culture to 141 rosettes when set in the field, where 7 plants died. Thus, the culture matured 134 plants, of which 20 were *nanella-brevistylis* and 114 *nanella*, a ratio of 1 : 5.7.

Culture 23.50 from the contents of 6 medium-sized capsules of the F_1 plant 21.29-2 produced 94 seedlings, of which 28 were stumpy; there were 377 sterile seeds, a poor germination of 20 percent. The plants set out numbered 55 and all of these matured, giving 10 plants of *nanella-brevistylis* and 45 plants of *nanella*, a ratio of 1 : 4.5.

The segregation of *nanella-brevistylis* from *nanella* was perfectly clear in both cultures, but the ratios of 1 : 5.7 and 1 : 4.5 showed the same departures from the expected ratio of 1 : 3 as appeared in my work with *brevistylis* and *Lamarckiana*. There was deficiency of *nanella-brevistylis*, and it seems probable that some factors work against the full expression of this segregate.

THE F_1 GENERATION OF *Lamarckiana*×*nanella-brevistylis*

The F_1 generation of *Lamarckiana*×*nanella-brevistylis* (culture 21.30) was through a plant (20.10-9) of my line of *Lamarckiana* from DE VRIES pollinated from the original plant of *nanella-brevistylis* (20.76-34). The contents of 1 capsule gave 114 seedlings, including 5 stumpy, and a residue of 126 sterile seeds, a germination of 47.5 percent. This was seed fertility distinctly higher than the line of *Lamarckiana* which, tested in the years 1916, 1917, 1919 and 1924, gave germinations, respectively, of 36, 36, 39 and 37 percent. Mortality among young plants and in the field permitted only 56 plants to mature, distributed as 34 *Lamarckiana*, 21 *nanella* and 1 *scintillans*. In no plant of this F_1 was there a suggestion of *brevistylis* characters, which were therefore completely recessive to the long-style complex.

The splitting of this F_1 generation into the two phenotypes, *Lamarckiana* and *nanella*, follows the same system as when crosses between *Lamarckiana* and *nanella* give in the F_1 large proportions of *nanella* plants among the *Lamarckiana* (DE VRIES 1913, p. 291), a performance which clearly indicates that genes for *nanella* are present in the heterozygous *Lamarckiana*. In this case, however, the *Lamarckiana* and *nanella*-like plants carry the recessive gene for the short style complex, as is proved by their performance in the F_2 generation.

Of this culture 4 plants *nanella*-like and 6 plants *Lamarckiana*-like were selfed, and their progeny in the F_2 will now be considered.

THE F_2 GENERATIONS FROM *Lamarckiana*×*nanella-brevistylis*

Culture 23.51 was from an F_1 *Lamarckiana*-like plant (21.30-1). The contents of 2 capsules gave 137 seedlings, of which 35 were stumpy, presenting at once a high mortality. There were 338 sterile seeds, a

TABLE 1

The F_2 generations from Lamarckiana×nanella-brevistylis.

CULTURE	APPEARANCE OF F_1 PARENT PLANT	SEEDLINGS	STERILE SEEDS	PERCENTAGE OF GERMINATION	*brevistylis*	*Lamarckiana*	*nanella-brevistylis*	*nanella*	OTHER FORMS	FAILED TO MATURE	STUNTED SEEDLINGS	DIED INCLUDING ALL STUNTED SEEDLINGS	RATIO OF SHORT-STYLED TO LONG-STYLED PLANTS
23.51	21.30–1 *Lamarckiana*	137 (2 capsules)	338	28.8	17	58	..	..	1 *oblonga* 2 *nanella*	1 *scintillans*-like rosette 8 *Lamarckiana*-like rosettes	35	50	1 : 3.6
23.52	21.30–2 *nanella*	86 (2 capsules)	172	33.3	..	..	24	44			4	18	1 : 1.8
23.53	21.30–3 *Lamarckiana*	119 (1 capsule)	206	36.6	15	81	..	..	1 hero (short-styled) 1 *nanella*	4 *Lamarckiana*-like rosettes	9	17	1 : 5.1
23.54	21.30–4 *nanella*	127 (1 capsule)	146	46.5	..	..	18	77			11	32	1 : 4.3
23.55	21.30–5 *nanella*	96 (2 capsules)	179	34.9	..	..	18	63	1 narrow-leaved dwarf (short-styled)	1 *nanella*-like rosette	6	13	1 : 3.3
23.56	21.30–9 *Lamarckiana*	115 (1 capsule)	232	33.1	9	70	..	..			11	36	1 : 7.8
23.57	21.30–13 *Lamarckiana*	129 (1 capsule)	233	35.6	20	83	..	..		3 *scintillans*-like rosettes 2 *Lamarckiana*-like rosettes	7	21	1 : 4.1
23.58	21.30–16 *Lamarckiana*	91 (1 capsule)	171	34.7	13	51	..	..	1 *nanella-brevistylis*	1 *scintillans*-like rosette	7	25	1 : 3.6
23.59	21.30–17 *Lamarckiana*	98 (1 capsule)	164	37.4	13	68	..	..	1 *nanella*	2 *scintillans*-like rosettes 1 *Lamarckiana*-like rosette	5	13	1 : 5.3
23.60	21.30–94 *nanella*	138 (2 capsules)	273	33.6	..	..	20	60		2 narrow-leaved dwarf rosettes 1 broad-leaved dwarf rosette	18	55	1 : 3

germination of 28.8 percent. Further mortality and the fact that 8 plants remained as rosettes reduced the number of plants that reached maturity to 78, distributed as 17 *brevistylis*, 58 *Lamarckiana*, 2 *nanella*, and 1 plant of *oblonga*. The proportion of *brevistylis* plants to all long-styled forms was then 17 to 61, a ratio of 1 : 3.6. (See table 1.)

Culture 23.52 was from an F_1 *nanella*-like plant (21.30-2). The contents of 2 capsules gave 86 seedlings, of which 4 were stumpy. There was a residue of 172 sterile seeds, a germination of 33.3 percent. Mortality reduced the culture at maturity to 68 plants, of which 24 were *nanella-brevistylis* and 44 *nanella*, a ratio of 1 : 1.8.

Culture 23.53 was from an F_1 *Lamarckiana*-like plant (21.30-3), one capsule of which gave 119 seedlings, including 9 stumpy, and a residue of 206 sterile seeds, a germination of 36.6 percent. There matured 98 plants distributed as 15 *brevistylis*, 1 robust short-styled plant with habit and foliage *gigas*-like, 81 *Lamarckiana* and 1 *nanella*. The short-styled plants were then as 16 to 82, a ratio of 1 : 5.1. The presence of the hero plant in this culture is a matter of interest. Its pollen was abundant but mostly shriveled. Unfortunately, the plant flowered too late in the season to make possible successful pollinations.

Culture 23.54 was from an F_1 *nanella*-like plant (21.30-4), one capsule of which gave 127 seedlings, including 11 stumpy, and 146 sterile seeds, a germination of 46.5 percent. Of the 95 plants which matured, 18 were *nanella-brevistylis* and 77 *nanella*, a ratio of 1 : 4.3.

Culture 23.55 was from an F_1 *nanella*-like plant (21.30-5). The contents of 2 medium-sized capsules gave 96 seedlings, including 6 stumpy, and 179 sterile seeds, a germination of 34.9 percent. There matured 82 plants distributed as 18 *nanella-brevistylis*, 1 narrow-leaved dwarf, short styled plant, and 63 *nanella*. The proportion of short styled plants to *nanella* was as 19 to 63, a ratio of 1 : 3.3.

Culture 23.56 was from an F_1 *Lamarckiana*-like plant (21.30-9), one capsule of which gave 115 seedlings, including 11 stumpy, and 232 sterile seeds, a germination of 33.1 percent. Heavy mortality reduced the number of plants that reached maturity to 79, of which 9 were *brevistylis* and 70 *Lamarckiana*, a ratio of 1 : 7.8.

Culture 23.57 was from an F_1 *Lamarckiana*-like plant (21.30-13). The contents of 1 capsule gave 129 seedlings, including 7 stumpy, and 233 sterile seeds, a germination of 35.6 percent. There remained in the field 3 rosettes *scintillans*-like and 2 rosettes *Lamarckiana*-like. There matured 103 plants of which 20 were *brevistylis* and 83 *Lamarckiana*, a ratio of 1 : 4.1.

Culture 23.58 was from an F_1 *Lamarckiana*-like plant (21.30-16) one capsule of which gave 91 seedlings, including 7 stumpy, and 171 sterile seeds, a germination of 34.7 percent. A *scintillans*-like rosette remained in the field. There matured 13 plants of *brevistylis*, 1 plant of *nanella-brevistylis* and 51 plants of *Lamarckiana*. The proportion of short-styled plants to *Lamarckiana* was then as 14 to 51, a ratio of 1 : 3.6. Of especial interest was the plant of *narella-brevistylis*, giving another instance of *brevistylis* characters passing over to a *nanella* plant from an immediate *Lamarckiana*-like parent heterozygous for *brevistylis* characters. This seems to be a case parallel to the appearance of the original plant of *nanella-brevistylis* (20.76-34) which came out in an F_2 from the cross *brevistylis* × *Lamarckiana*.

Culture 23.59 was from an F_1 *Lamarckiana*-like plant (21.30-17) one capsule of which gave 98 seedlings, including 5 stumpy, and 164 sterile seeds, a germination of 37.4 percent. There remained as rosettes 2 *scintillans*-like and 1 *Lamarckiana*-like. There came to maturity 83 plants distributed as 13 *brevistylis*, 68 *Lamarckiana* and 1 *nanella*. The ratio of *brevistylis* to long-styled plants was as 13 to 69, or 1 : 5.3.

Culture 23.60 was from an F_1 *nanella*-like plant (21.30-94). The contents of 2 medium-sized capsules gave 138 seedlings, including 18 stumpy, and 273 sterile seeds, a germination of 33.6 percent. There remained in the field 2 narrow-leaved dwarf rosettes and 1 broad-leaved dwarf rosette. Heavy mortality reduced the number of plants that matured to 80, distributed as 20 *nanella-brevistylis* and 60 *nanella*, a ratio of 1 : 3.

The results from the ten cultures of the F_2 generations from *Lamarckiana* × *nanella-brevistylis*, as given above, are presented in table 1. Certain general conclusions stand out clearly. The segregation of short-styled plants from long-styled was absolutely clear, there being no intergrades for any of the characters that make up the short-style complex. The proportion of short styles to long was very close to the monohybrid ratio of 1 : 3 in four of the cultures (23.51, 55,58,60), ranging from 1 : 3 to 1 : 3.6. In five cultures the ratios ranged from 1 : 4.1 to 1 : 7.8, thus presenting smaller proportions of short-styled plants than the ratio 1 : 3. In one culture (23.52) the ratio of 1 : 1.8 showed a larger proportion of short-styled plants than was to be expected. Thus, in general, these results agreed with those of my earlier studies in that there appear to be factors working against the full numerical expression of the short-styled segregates.

As was to be expected, the F_2 cultures from the F_1 *nanella*-like plants gave only dwarf plants. The cultures from the F_1 *Lamarckiana*-like

plants threw occasional variants such as *nanella*, *oblonga*, a hero type with short styles, *scintillans*-like rosettes, and a narrow-leaved short-styled dwarf. In one culture (23.58) a *nanella-brevistylis* again appeared from an F_1 *Lamarckiana*-like parent heterozygous for *brevistylis* characters.

The class of stumpy seedlings appeared in every culture and sometimes in large numbers and materially raised the totals of plants that died during the season. Stumpy seedlings, since they develop no roots, live only a few days. The other mortality, as usual, was distributed throughout the season, but most largely affected plants in earlier stages of development.

COMPARATIVE GROWTH RATES OF POLLEN TUBES CARRYING FACTORS FOR SHORT AND LONG STYLES, RESPECTIVELY

Following the method employed by HERIBERT-NILSSON (1920), a test was made of the comparative growth rates of pollen tubes carrying genes for short and long styles, respectively. The procedure was to cut off the styles of flowers from an F_1 plant, close to the ovaries, at various time intervals following pollination. If pollen tubes carrying genes for short styles should grow more slowly than those with genes for long styles it should be possible to obtain ratios in the F_2 with larger proportions of long-styled plants than usual if the operation was performed at a time before many short-styled pollen tubes had reached the ovary. The plant selected for the experiment was the *Lamarckiana*-like F_1 plant 20.30-1, *Lamarckiana*×*nanella-brevistylis*, normally developed capsules of which gave culture 23.51 in table 1.

As shown in table 2, no fertilizations were effected until 22 hours after pollination, although attempts were made to obtain seed 17, 18, 19, 20 and 21 hours after pollination. Four attempts to obtain seed after 22 hours were failures, but 7 small capsules about 12 mm long were obtained from other trials. Larger capsules, 14 mm long, were produced 23 hours after pollination, and still larger, 15 to 16 mm long, when the interval between pollination and the cutting of the style was 24 hours. The ratio of short to long styles following a normal withering of the flowers on this plant was given by culture 23.51. The results of the experiment were as follows.

Culture 23.81 was from 7 capsules, 12 mm long, developed 22 hours after pollination. The harvest gave 54 seedlings, including 5 stumpy, and there was a residue of 204 sterile seeds, a germination of 20.9 percent. An *oblonga*-like rosette, a *scintillans*-like rosette and a *Lamarckiana*-like rosette remained in the field. There matured 5 plants of *brevistylis* and 25 plants of *Lamarckiana*, giving proportions of short-styled to long-styled in the ratio of 1 : 5.

Culture 23.82 was from 3 capsules, 14 mm long, developed 23 hours after pollination. There were 68 seedlings, including 4 stumpy, and 238 sterile seeds, a germination of 22.2 percent. There remained in the field 2 *Lamarckiana*-like rosettes. There matured 8 plants of *brevistylis* and 40 plants of *Lamarckiana*, a ratio of 1 : 5.

Culture 23.83 was from 4 capsules, 15 to 16 mm long, developed 24 hours after pollination. These gave 101 seedlings, including 7 stumpy, and 406 sterile seeds, a germination of 19.9 percent. There remained in the field 2 *oblonga*-like rosettes and 3 *Lamarckiana*-like. There matured 20 plants of *brevistylis*, 1 plant of *nanella-brevistylis*, 58 plants of *Lamarckiana* and 1 plant of *nanella*. The proportion of short-styled plants to long-styled was then as 21 to 59, a ratio of 1 : 2.8. We note again, as in culture 23.58, table 1, the appearance of a *nanella-brevistylis* from an F_1 *Lamarckiana*-like plant heterozygous for *brevistylis* characters.

For purpose of comparison with the results of the experiments from cultures 23.81, 23.82 and 23.83, there is given at the bottom of table 2 the data from culture 23.51 repeated from table 1. Culture 23.51 was from seeds of the same plant, 20.30-1, on which the experiments were performed, but its flowers withered normally and consequently the pollen tubes had the full time to accomplish all fertilizations possible.

A comparison of the four cultures (see table 2) offers the following conclusions. (1) With increasing length of time for the growth of pollen tubes there are produced higher average numbers of seeds per capsule (27, 102, 127 and 237 seeds), and a corresponding increase in the size of the capsules (12, 14, 15, 16 and 21 mm). (2) The percentages of viable seeds are lower when full fertilization is prevented (20.9, 22.2, 19.9 and 28.8 percents of germination). This is probably due to less efficient nutrition of the smaller capsules. (3) The ratio of short-styled to long-styled plants in the F_2 was lower (1 : 5) when the time interval for the growth of pollen tubes was 22 and 23 hours. The ratio (1 : 2.8) was close to expectations when the time interval was 24 hours, agreeing closely with the ratio (1 : 3.6) from capsules following a normal withering of the flowers.

Although the data are not extensive, nevertheless they suggest that pollen tubes carrying the genes for the short-style complex do not grow as rapidly as those with genes for the long-style complex. Long-styled pollen tubes apparently reach the ovary earlier and may be expected to effect the fertilization of a larger proportion of the ovules than would otherwise take place, a result expressed by lower proportions of *brevistylis* segregates. The difference in rate of growth is not great, since a time inter-

TABLE 2

The F_2 generations after various time intervals permitted for the growth of pollen tubes down the style. Experiments with the F_1 Lamarckiana-like plant, 21.30–1, of the cross Lamarckiana×nanella-brevistylis. Styles were cut close to the ovaries after the time intervals following pollination as given in the first column.

TIME INTERVAL FOR GROWTH OF POLLEN TUBES	NUMBER OF FLOWERS POLLINATED	NUMBER OF CAPSULES DEVELOPED	LENGTH OF CAPSULE	AVERAGE NUMBER OF SEEDS	CULTURE	SEEDLINGS	STERILE SEEDS	PERCENTAGE OF GERMINATION	*brevistylis*	*Lamarckiana*	OTHER FORMS	FAILED TO MATURE	STUMPY SEEDLINGS	DIED INCLUDING ALL STUMPY SEEDLINGS	RATIO OF SHORT-STYLED TO LONG-STYLED PLANTS
17 hours 18 hours 19 hours 20 hours 21 hours 22 hours	3 4 6 5 3 4	none none none none none none													
22 hours	7	7	12 mm	37	23.81	54	204	20.9	5	25		1 *oblonga*-like rosette 1 *scintillans*-like rosette 1 *Lamarckiana*-like rosette	5	21	1 : 5
23 hours	3	3	14 mm	102	23.82	68	238	22.2	8	40		2 *Lamarckiana*-like rosettes	4	18	1 : 5
24 hours	4	4	15 to 16	127	23.83	101	406	19.9	20	58	1 *nanella-brevistylis* 1 *nanella*	2 *oblonga*-like rosettes 3 *Lamarckiana*-like rosettes	7	16	1 : 2.8
A normal withering of the flower	2	2	21 mm	237	23.51	137	338	28.8	17	58	1 *oblonga* 2 *nanella*	1 *scintillans*-like 8 *Lamarckiana*-like rosettes	35	50	1 : 3.6

val of 24 hours gives the short-styled pollen tubes an opportunity to record their presence by a ratio approximating the monohybrid 1 : 3.

It is admitted that conclusions of this character must be taken with due allowance for the difficulties of experimentation. The principal weakness lies in the loss of plants through death, including the large group of stumpy seedlings. We should very much like to know the genetical constitution of the stumpy seedlings. However, these results are presented as pointing towards the sort of experimental work necessary to solve some of the problems presented by seeming departures of Oenothera genetics from Mendelian principles. The writer has no sympathy with a group of Oenothera workers which has held that Oenothera genetics follows a system apart from the fundamentals of Mendelism which is an orderly process of segregation from heterozygous stock. In Oenothera material orderly segregation is present but usually in complex relations for specific reasons which research should endeavor to determine.

DISCUSSION

My earlier studies (DAVIS 1918, 1921) showed that malnutrition of developing seeds on F_1 plants in crosses between *brevistylis* and *Lamarckiana* will kill a larger proportion of *brevistylis* zygotes or embryos than of *Lamarckiana*. As a result *brevistylis* segregates in the F_2 and in backcrosses of the F_1 to the *brevistylis* parent will be in smaller proportions than the expected 1 : 3 and 1 : 1 ratios. The experiments here recorded on rate of pollen-tube growth indicate that pollen tubes with genes for short styles grow more slowly than do those with genes for long styles. Thus, we seem to have discovered two factors which work against the full expression of *brevistylis* segregates from crosses involving plants heterozygous for *brevistylis* characters. The facts of segregation are unmistakable; the ratios clearly indicate the Mendelian monohybrid system; the departures from expectations are due to factors which operate to the disadvantage of *brevistylis* segregates.

Some geneticists may be tempted to interpret these cases as tied up with lethals, but I can see no justification for such considerations. The death of zygotes or embryos of *brevistylis* in competition with those of *Lamarckiana* under conditions of limited nutrition is a phase in the struggle for existence. The apparently slower growth of pollen tubes carrying genes for *brevistylis* is a matter of physiology, but it is serious to the species in that it must lead to lower proportions of *brevistylis* fertilizations when there are present a larger number of pollen tubes with genes for long styles than there are ovules to be fertilized.

What may be the interpretation of the facts of dwarf and of short-style segregation from the crosses here discussed? We have the following basis on which to build genetical formulae: (1) *Brevistylis* characters are recessive to those of *Lamarckiana*, and short styles when present in *nanella* (*nanella-brevistylis*) are recessive to typical *nanella*. (2) *Nanella* breeds true, but *Lamarckiana* throws its small percentage of *nanella* plants. (3) *Lamarckiana*×*brevistylis* gives about the same proportion of *nanella* as *Lamarckiana* selfed (DE VRIES 1901), showing that *brevistylis* is also heterozygous for tall stature. (4) *Nanella* crossed to a number of Oenothera species (DE VRIES 1913, p. 292) gives no dwarfs in the F_1, but dwarfs appear in the F_2, which indicates that dwarfness is recessive. (5) *Nanella* crossed to *Lamarckiana* gives *nanella* in fair proportions with *Lamarckiana* (DE VRIES 1913, p. 207), which implies that *Lamarckiana* carries *nanella* genes as recessives. (6) *Nanella* pollinated by *brevistylis* is reported (DE VRIES 1913, p. 207) to give *nanella* in proportions of 31 to 50 percent, again indicating that *brevistylis* also carries *nanella* genes as recessives. The *brevistylis* characters do not appear in the F_1 of this cross (DE VRIES 1913, p. 190) but come out in the F_2. (7) The small percentages of *nanella* from selfed *Lamarckiana* may be due to conditions which permit only an occasional successful union of gametes carrying the recessive genes to form zygotes homozygous for dwarfness, or which permit only occasionally the survival and development of such zygotes. There are sufficient degrees of sterility, both gametic and zygotic, to permit of either possibility.

Let *T* represent the gene for the stature of *Lamarckiana* and *brevistylis* and *t* its allelomorph for the recessive dwarfness of *nanella*. Let *L* stand for the long style of *Lamarckiana* and *nanella* and *l* for the recessive short style of *brevistlyis* and *nanella-brevistylis*. Then, if the above data are correct, and if the genes for stature and for style length are in different chromosomes, we may assume the formula of *Lamarckiana* to be *TtLL*, *brevistylis Ttll*, *nanella ttLL*, and *nanella-brevistylis ttll*. Should the genes for stature and style length be in the same chromosome, the formula for *Lamarckiana* would be *TLtL*, *brevistylis Tltl*, *nanella tLtL*, and *nanella-brevistylis tltl*.

There are then two sets of hypothetical genetical formulae which must be tested in their application to the breeding data presented in this paper. The first set places the genes for stature and for style length in different chromosomes; the second set assumes that these genes are linked in the same chromosome. The origin of the first plant of *nanella-brevistylis* in the F_2 of the cross *brevistylis*×*Lamarckiana* is permitted by either set of

formulae. Both sets of formulae allow the segregation of the short-style complex in the F_2 generations from the cross *nanella*×*nanella-brevistylis* and from the F_1 *nanella*-like plants of the cross *Lamarckiana*×*nanella-brevistylis*. The test of the merits of the two sets of genetical formulae comes with their application to the breeding behavior of the F_1 *Lamarckiana*-like plants from the cross *Lamarckiana*×*nanella-brevistylis*.

By the second set of formulae (assuming linkage of the genes for stature and style length in the same chromosome) the cross *Lamarckiana* (*TLtL*) ×*nanella-brevistylis* (*tltl*) would give in the F_1 *Lamarckiana*-like plants (*TLtl*) and *nanella*-like plants (*tLtl*). The *Lamarckiana*-like F_1 plants (*TLtl*), selfed, should by this hypothesis segregate these genes as 1 *TLTL* : 2 *TLtl* : 1 *tltl*, which would be 3 *Lamarckiana*-like plants to 1 *nanella-brevistylis*. The facts are that such plants (tables 1 and 2) give *brevistylis* and *Lamarckiana* in ratios close to 1 : 3, together with occasional dwarfs.

By the first set of formulae (assuming the genes for stature and style length to lie in different chromosomes) the cross *Lamarckiana* (*TtLL*) ×*nanella-brevistylis* (*ttll*) would give in the F_1 *Lamarckiana*-like plants (*TtLl*) and *nanella*-like plants (*ttLl*). The *Lamarckiana*-like plants (*TtLl*) being dihybrids would give, if segregation were fully expressed: 1 *TTLL*, 2 *TTLl*, 2 *TtLL*, 4 *TtLl*, 1 *TTll*, 2 *Ttll*, 1 *ttLL*, 2 *ttLl*, 1 *ttll*. This is a distribution of 9 *Lamarckiana*-like : 3 *brevistylis*-like : 3 *nanella*-like : 1 *nanella-brevistylis*. These segregates all appear, as is shown from cultures 23.51-53-56-57-58-59 of table 1, and cultures 23.81-82-83 and 23.51 (repeated) of table 2, which thus satisfy the formulae that assume genes for stature and style length to lie in different chromosomes.

Thus, expectations are realized as to the character of the segregates from the *Lamarckiana*-like plants, but there is the same deficiency of dwarfs characteristic of the progeny from *Lamarckiana*. The total of dwarfs (*nanella* and *nanella-brevistylis*) from cultures 23.51-53-56-57-58-59 of table 1 is only 5 plants in contrast to 498 tall plants (*Lamarckiana* and *brevistylis*); they make up 1 percent of the cultures, which is close to the percentage of *nanella* from *Lamarckiana* as reported by DE VRIES.

Of the full set of theoretically possible segregates from the F_1 *Lamarckiana*-like plants listed above, it is possible that those homozygous for tallness (*TT*) never develop, since we know of no lines of *Lamarckiana* and *brevistylis* homozygous for tall stature. If this is so, we may take from the list the plant *TTLL*, the 2 plants *TTLl* and the plant *TTll*, and the proportions of the four types would become 6 *Lamarckiana*-like : 2 *brevistylis*-like : 3 *nanella* like : 1 *nanella-brevistylis*. The ratio of short-

styled plants to long-styled would remain as before, 1 : 3, but the ratio of dwarfs to tall would be 1 : 2.

Let us apply MULLER'S (1918) hypothesis of balanced lethals to the situation presented by the small percentage of dwarf segregates from *Lamarckiana* in selfed lines and from the F_1 *Lamarckiana* plants of the cross *Lamarckiana* × *nanella-brevistylis*. We assume for the chromosomes carrying the genes for high stature, *T*, and for dwarfness, *t*, two different lethals, v_1 and v_2. The formula for *Lamarckiana* would then be Tv_1tv_2LL, for *brevistylis*, Tv_1tv_2ll, for *nanella*, tv_1tv_2LL, and for *nanella-brevistylis*, tv_1tv_2ll. If from these hypothetical formulae the segregation is worked out for *Lamarckiana* and *nanella* selfed, and from the crosses between *Lamarckiana* and *nanella*, between *Lamarckiana* and *nanella-brevistylis* and between *nanella* and *nanella-brevistylis*, it will be found that certain classes of segregates will carry v_1v_1 or v_2v_2 and others the combination v_1v_2. It is assumed that the classes homozygous for either lethal (v_1v_1 or v_2v_2) fail to develop and that only such plants live as are heterozygous for both lethals (v_1v_2). The low seed fertility of *Lamarckiana*, *brevistylis*, *nanella* and *nanella-brevistylis*,—less than 50 percent,—permits this hypothesis.

The character of the segregation of long and short styles is not affected by the assumptions involved in the hypothesis of balanced lethals. The hypothesis will, however, only permit of the appearance of dwarfs from *Lamarckiana* or from the F_1 *Lamarckiana* plants of the cross *Lamarckiana* × *nanella-brevistylis* on the assumption of repeated and regular crossings over between the genes for stature and the lethals. Such crossing over would give dwarf plants, the ratio of which would be determined by the frequency of the crossing over. The hypothesis of balanced lethals is then concerned with a special modification of segregation to give dwarfs by crossing over and does not admit a problem of deficiency. For this reason it is much simpler than an hypothesis which allows a regular segregation of dwarfs, but must explain why they fall so very far short of the expected proportions.

I have tried to find evidence for some simple differential that would account for the small proportions of dwarfs without involving the assumption of lethals. Thus, if pollen tubes from pollen grains carrying genes for dwarfness grew more slowly than those from pollen grains with genes for tall stature there would be a physiological factor working against the appearance of dwarfs whenever there were many more pollen tubes present than ovules to be fertilized. Under such conditions the segregation from an F_1 *Lamarckiana*-like plant (*TtLl*) of the cross *Lamarckiana* × *nanella-brevistylis* might give only the tall plants 1 *TTLL*, 2 *TTLl*,

1 *TTll*, 1 *TtLL*, 2 *TtLl*, 1 *Ttll*, with perhaps an occasional dwarf. The data assembled in table 2, however, do not help us with the problem, even though the dwarfs did not come out in cultures 23.81 and 23.82, because the data are not sufficiently extensive to justify conclusions with the factor of probable error so large. It is possible that further experimentation may give clearer evidence of physiological factors to account for a deficiency of dwarfs, but as matters stand the hypothesis of balanced lethals is likely to appeal more strongly to the geneticist.

Finally, there is the hypothesis of mutation to account for the appearance of dwarfs from *Lamarckiana*. DE VRIES believed that *Lamarckiana* is pure for tall stature, which would mean that its formula would be *TT* and not *Tt* as assumed in this paper. The mutation of *T* to *t* in sufficient frequency would result in the production by *Lamarckiana* of gametes carrying *t*, which through union with one another would give *nanella*, *tt*. Thus, *Lamarckiana* in selfed line might be expected to throw *nanella* and when crossed to *nanella* would be expected to give a progeny of *Lamarckiana*-like forms, *Tt*, and *nanella*. The hypothesis assumes mutation of a frequency far beyond that which seems probable from the experience of geneticists in such thoroughly studied material as *Drosophila* and maize. It must assume that the *Lamarckiana*-like plants (*Tt*) coming out of the crosses with *nanella* are different from the parent *Lamarckiana*, of which there is no evidence, or that *t* mutates back to *T*. The regularity of the appearance of *nanella* from *Lamarckiana* and from the crosses between *nanella* and *Lamarckiana* will seem to the geneticist as much more likely to rest upon some expression of segregation. The fact that *nanella* crossed to certain other species of Oenothera does not give dwarfs, implies that *Lamarckiana* is not homozygous for tall stature. Finally, there is the accumulated evidence from various viewpoints, too numerous to be discussed here, which throws doubt on the genetical purity of *Lamarckiana* and of most Oenotheras that have been carefully studied, and leads us to believe that the genus is chiefly an assemblage of impure species. Attempts to establish evidence for mutation from such material must expect keen scrutiny and criticism from geneticists.

SUMMARY

Oenothera nanella-brevistylis is a dwarf with the habit and stature of *nanella* but with those characters of *brevistylis* which make up the short-style complex. The short-style complex consists of a short style with deformed or rudimentary stigma lobes, bud cones more stout and with short sepal tips, broader less pointed bracts, and ovary partly superior

and ripening few or no seeds. The plant first appeared in an F_2 from the cross *brevistylis*×*Lamarckiana* (DAVIS 1921, p. 580, table 3). This plant produced no seed when pollinated, and consequently crosses could be made with it only as the pollen parent.

The F_1 generation of *nanella*×*nanella-brevistylis* consists of plants uniformly *nanella*-like. Thus, the short-style complex of *nanella-brevistylis* is recessive to the long-style complex of *nanella*, as are the same characters in *brevistylis* recessive to those of *Lamarckiana*.

F_2 generations from two selfed F_1 plants of *nanella*×*nanella-brevistylis* presented plants uniformly dwarf but segregating sharply as having either the long-style complex of *nanella* or the short-style complex of *nanella-brevistylis*. The ratios of *nanella-brevistylis* to *nanella* were 1 : 5.7 (culture 23.49) and 1 : 4.5 (culture 23.50), thus departing from the expected monohybrid ratio of 1 : 3 with a deficiency of *nanella-brevistylis* probably due to some factors working against the full expression of this segregate. This deficiency of *nanella-brevistylis* agreed with a similar deficiency of *brevistylis* segregates in the F_2 of its cross with *Lamarckiana* (DAVIS 1918, 1921).

The F_1 generation of *Lamarckiana*×*nanella-brevistylis* consisted of 30 plants *Lamarckiana*-like, 21 plants *nanella*-like and one plant *scintillans*-like. There was no evidence of the short-style complex in this generation, showing it to be completely recessive to the long-style complex.

F_2 generations from *Lamarckiana*×*nanella-brevistylis* were grown from the seed of 4 *nanella*-like and 6 *Lamarckiana*-like plants of the F_1. The F_2 generations from the selfed *nanella*-like F_1 plants (table 1) consisted of *nanella-brevistylis* and *nanella* in ratios of 1 : 1.8, 1 : 3, 1 : 3.3 and 1 : 4.3 (cultures 23.52, 60, 55, 54). The F_2 generations from the selfed *Lamarckiana*-like F_1 plants (table 1) consisted of *brevistylis* and *Lamarckiana* in ratios of 1 : 3.6, 1 : 3.6, 1 : 4.1, 1 : 5.1, 1 : 5.3 and 1 : 7.8 (cultures 23.51, 58, 57, 53, 59, 56). There was a scattering of variants in the cultures from the F_1 *Lamarckiana*-like plants (table 1) including one short-styled hero plant (culture 23.52) and one *nanella-brevistylis* (culture 23.58). The segregation of the short-style complex from the long-style was perfectly sharp; but as in my earlier studies on crosses between *brevistylis* and *Lamarckiana* (DAVIS 1918, 1921), there was generally a smaller proportion of *brevistylis* segregates than the expected 1 : 3 ratio would require.

Experiments on comparative growth rate of pollen tubes on a selected F_1 plant (21.30-1, *Lamarckiana*×*nanella-brevistylis*) carrying genes for the short- and long-style complexes showed (table 2): (1) That no fertiliza-

tion was effected until about 22 hours after pollination. (2) That small capsules were developed 22 hours after pollination, the seed of which gave an F_2 generation with the proportions of *brevistylis* to *Lamarckiana* in the ratio of 1 : 5 (culture 23.81). (3) That somewhat larger capsules, developed 23 hours after pollination, gave an F_2 with *brevistylis* and *Lamarckiana* in the ratio also of 1 : 5 (culture 23.82). (4) That still larger capsules developed 24 hours after pollination gave *brevistylis* to *Lamarckiana* in the ratio of 1 : 2.8 (culture 23.83). In this culture appeared the third plant of *nanella-brevistylis*. Capsules on the same F_1 plant, developed after a normal withering of the flowers, gave an F_2 with the proportions of *brevistylis* to *Lamarckiana* in the ratio of 1 : 3.6. These data, although not extensive, suggest that pollen tubes carrying genes for the short-style complex do not grow as rapidly as do those with genes for the long-style complex. Thus, a factor has perhaps been discovered which may operate to lower the proportions of short-styled segregates.

From my earlier work (DAVIS 1921) and the experiments recorded above we may have established two factors which work to the disadvantage of short-styled segregates: first, malnutrition of zygotes and embryos; and second, slower growth rate of pollen tubes carrying genes for the short-style complex. The results of such operations would be smaller proportions of short-styled segregates than theory would indicate, which seems to agree with the facts.

From the breeding data given in the discussion it is clear that the genes for stature (*T* and *t*) and for style length (*L* and *l*) are in different chromosomes as suggested by SHULL (1921). Therefore, with respect to the relation of these genes to one another the formula for *Lamarckiana* is *TtLL*, *brevistylis*, *Ttll*, *nanella*, *ttLL* and *nanella-brevistylis*, *ttll*. By these formulae the segregation of the short-style complex from this material should follow the monohybrid ratios, which agrees with the facts except that, as noted above, the proportion of short-styled segregates is somewhat smaller than expectation. The formulae also permit the appearance of the double recessive *nanella-brevistylis*, from appropriate crosses.

There is a constant and marked deficiency of dwarfs from *Lamarckiana*, from *brevistylis*, and from crosses involving these two types. In the absence of evidence for some differential working against the appearance of dwarfs, MULLER'S hypothesis of balanced lethals offers the most satisfactory interpretation for this breeding behavior.

Let v_1 and v_2 stand for two lethals in the chromosomes concerned with stature, and the formulae become for *Lamarckiana*, Tv_1tv_2LL, for *brevistylis*, Tv_1tv_2ll, for *nanella*, tv_1tv_2LL, and for *nanella-brevistylis*, tv_1tv_2ll. Crosses

involving these formulae will give classes of segregates carrying v_1v_1 or v_2v_2, and others with the combination v_1v_2. It is assumed that the classes homozygous for either lethal (v_1v_1 or v_2v_2) fail to develop, and that only such plants live through the fertile seeds as are heterozygous for both lethals (v_1v_2). The proportions of fertile seeds,—under 50 percent,—in *Lamarckiana*, *brevistylis*, *nanella* and *nanella-brevistylis*, permit this assumption of zygotic lethals. On the further assumption of repeated and regular crossings over between the genes for stature and the lethals, dwarfs would appear in proportions determined by the crossover frequency. The regularity of the segregation of the short-styled complex in a different chromosome is not affected by these lethals associated with the chromosome carrying genes for stature.

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THE PRECIPITIN REACTION IN RELATION TO GRAFTING[1]

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INTRODUCTION

Since it is apparent that one of the principal factors for the successful production of grafts, and possibly also for hybridization, is that the plants should be nearly related, and since the relationship among animals has been successfully studied by means of immunity reactions, I have tried to employ these more exact and scientific methods in the investigation of plants. For this purpose, I have used what is known as the "precipitin reaction."

This reaction, discovered by KRAUS in 1897, gave to UHLENHUTH (1900, 1901), WASSERMANN (1903) and NUTTALL (1904) the means of differentiating the various species of animals through their specific proteins. As precipitins are formed with any soluble animal or vegetable protein, they were made use of also in botany. The phytoprecipitins, sa they are called inbotany, were first studied by KOWARSKY (1901), MAGNUS and FRIEDENTHAL (1906), MAGNUS (1908), GASIS (1908) and others. These authors proved that specific precipitins could be obtained from various plants, but did not attempt a methodical investigation in this field, as had been done with animals.

In 1913 the Botanical School of Königsberg, under the direction of MEZ, started methodical researches for the classification of plants by

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means of the precipitins. This school, guided by the previous classifications based on the morphological and structural appearances of plants, started to test them by means of this immunological reaction (GOHLKE 1913), and, although the new classification is still in its infancy, they have already examined a large number of plants. MEZ and GOHLKE (1913) have studied the Angiosperms; MEZ and LANGE (1914) the Ranales; MEZ and PREUSS (1914) the Parietales; MEZ and KIRSTEIN (1920) the Gymnosperms; SALTZMANN (1924) has reviewed once more the Ranales and completed the Gnetales; RAEDER (1924) the Adoxaceae, Loranthaceae, Ericaceae, Polygalaceae, Empetraceae and Hypericaceae; GUTTMANN (1924) the Archegoniatae; STEINECKE (1925) the genealogy of the Algae. This work, of the most interesting nature on account of its fundamental character, is not yet generally acknowledged, but, as RAEDER says, "the genealogy of plants established by means of the sero-diagnostic reactions is gaining more and more recognition."

As the literature seems to be mute on the subject of immunological reaction in relation to grafting among plants, I undertook a study of the behavior of the precipitin reaction in relation to a few plants known to be successfully intergrafted. The external characteristics are not an absolutely reliable rule by which one can judge the degree of the internal and constitutional relationship either for the vegetative or for the sexual affinity between the different species.

The object of this research was to investigate whether plants having been classified in families by their morphological characteristics and generally known to be capable of being intergrafted, would react in the same way to the specific biological reaction of the precipitins.

The plants which I have particularly studied are the following:

(1) Family of the Rutaceae; Genus: Citrus.
- *Citrus aurantium* (orange).
- *Citrus limonum* (lemon).
- *Citrus vulgaris* (bitter orange).
- *Citrus decumana* (grapefruit).
- *Citrus nobilis* (mandarin orange of China).

(2) Family of the Rosaceae; Sub-family: Pomoideae.
- *Pyrus communis* (pear).
- *Pyrus malus* (apple).

(3) Family of the Rosaceae; Sub-family: Prunoideae.
- *Prunus domestica* (plum).
- *Prunus armeniaca* (apricot).
- *Prunus persica* (peach).
- *Prunus amygdalus* (almond).

(4) Family of the Solanaceae.
Solanum tuberosum (potato).
Lycopersicum esculentum (tomato).
(5) Family of the Chenopodiaceae.
Beta vulgaris (beet).
(6) Family of the Polygonaceae.
Rheum officinale (rhubarb).
(7) Family of the Oleaceae.
Olea europaea (olive).

The last three plants mentioned were used principally as controls.

TECHNIQUE

Preparation of the extract

The extract may be prepared from any part of the plant under investigation. I prepared all the extracts from the seeds, which, as previously noted by others, owing to the small amount of water and the large amount of soluble albumins contained in them, give an all-round greater uniformity of the extracts. The dried seeds, preferably of the same season, are reduced to a fine powder by means of a coffee grinder or, if the seeds are too large, by means of a chopping machine. The powder is freed from chaff by sifting and 5 grams of the fine flour-like powder is put into a perfectly clean and sterile Erlenmeyer flask with 50 cc of sterile 0.9 percent NaCl solution. The mixture is kept at room temperature for 12 hours and is occasionally vigorously shaken. It is then poured onto 6 layers of sterile gauze and well pressed. The milky filtrate thus obtained is then filtered twice through filter paper, Schleicher and Schüll No. 595. A sample of the clear filtrate is now taken and its albumin content is estimated by means of an Esbach albuminometer, using the Tsuchiya reagent (phosphotungstic acid 1.50 gm; hydrochloric acid 5 cc; alcohol 95 percent, 95 cc). The reading was taken after 2 hours and the amount of albumin per liter noted. As a rule, the extracts thus prepared averaged an albumin content of 3 grams per liter. As I intended to work with extracts containing approximately the same amount of albumin, if the extract did not conform to that definite amount of albumin, a correction was necessary. This correction was made, naturally, either by increasing the amount of seed flour or by diluting the extract proportionally. I laid great stress on this special point of technique in my research. The main portion of the filtrate was incubated at 60° C for one hour, after which time, it was transferred to another incubator at 37°C for 24 hours. This

fractional sterilization was repeated for 3 consecutive days. Afterwards, the extracts were preserved in the ice-chest until required for use. No chemicals were used as preservatives. As a rule, all extracts thus prepared and treated showed a certain amount of precipitate due, probably, to the temperature and the acid reaction of the fluid. Although this coincidence probably diminishes the contents of active substances, it does not seem to interfere with the efficacy of the extract. Extracts which, notwithstanding all the precautions taken, gave rise to moulds, were discarded.

SALTZMANN (1924) prepared the extracts from dried and powdered leaves and to test the presence of albumin in the extracts he used GUTTERMANN'S method of demonstrating the presence of organic substances, using the Berlin bleu reaction for nitrogen and the nitroprussiate reaction for detecting the presence of sulphur. PREUSS and SALTZMANN have had good results in preparing the extract, the former with 0.1 percent and the latter with 0.5 percent NaOH.

Immunization

The animal used for immunization was the rabbit. Brown male rabbits of an average weight of 3000 grams were chosen. Before and during the process of immunization, they were fed only on carrots.

A precise neutralization of the extract previous to injecting the animal to be immunized is of capital importance. I used sodium hydroxide 0.1 percent and acetic acid 0.1 percent, using phenolphtalein as indicator.

With all the precautions of asepsis, 10 cc of carefully neutralized extract are injected into the peritoneum of the animal. The intravenous way may also be used but the intraperitoneal is safer, as, given the slow absorption of the material, it causes less reaction. The latter way is imperative, however, when immunizing animals with extracts prepared from seeds containing large amounts of toxic substances like solanin, nicotin, etc., in which case the process of immunization must be carried out with very great care and appropriate dosage. An interval of 4 days elapses between successive injections. The number of injections given averages from 7 to 10. A total quantity of 120 to 140 cc is injected, the first and last 2 injections being of 20 cc each and the intervening injections of 10 cc each. Rabbits, as a rule, bear the injections very well. Although at the beginning of the immunization they show nc loss in weight, a loss is noticeable in the last few weeks of immunization.

A preliminary test of the serum of the animal so treated, when made to react with the extract used for immunization diluted in convenient proportions, will show, by the formation of a well-defined precipitate,

the titer of the serum. The highest titer of my immune sera, as a rule, was between 6400 and 12,800. If the immune serum is found to give rise to a satisfactory precipitate, the animal is starved for 24 hours and then sacrificed by collecting the blood from one of the carotids, with all the precautions of asepsis, in a sterile Erlenmeyer. The blood is preserved in the ice-chest until the day after, when it is centrifuged; the supernatant clear serum is pipetted off and transferred to 5 cc glass ampoules, sealed at the flame without adding any preservatives and put in a dark, cool place.

The precipitin reaction

Both in performing the test and in preparing the reagents, I was guided by the details of technique given by UHLENHUTH, NUTTALL and the school of MEZ, and followed scrupulously those which I learned personally from WASSERMANN. In my researches, I have adopted the method of adding a constant amount of immune serum to a series of progressively diminishing dilutions of precipitinogen.

A series of 12 test tubes (7 cm in length and 1 cm in diameter) is laid in a row in a rack. The first 10 tubes are marked progressively from 50 to 25,600, meaning dilutions from 1 in 50 to 1 in 25,600. The eleventh is marked "E" for extract; the twelfth, which contains a solution of 0.9 percent sodium chloride, is marked NaCl. A second series of tubes, marked exactly like the first, is put in the second row of the rack. This second series represents the control. In tubes No. 2 to No. 11 of both series 0.5 cc of absolutely clear filtered and sterile 0.9 percent NaCl solution is put. With a sterile 1 cc pipette, 0.5 cc of very carefully neutralized and absolutely clear extract is put in tubes No. 1 and No. 11. Another 0.5 cc of the same extract is put in tube No. 2 of the front row of tubes. From tube No. 2, which now contains 1 cc of fluid, after well mixing the extract with the NaCl by sucking and expelling the fluid with the pipette, 0.5 cc is aspirated and transferred to tube No. 3. The same procedure is repeated up to tube No. 10, from which the extra 0.5 cc of the fluid is discarded. Thus, each tube in the first row now contains 0.5 cc of fluid as specified above. In the second row of tubes, the same dilutions of precipitinogen are repeated in exactly the same way as in the first. To each tube of the first row, with the exception of No. 11, is added 0.1 cc of the immune serum prepared against the homologous extract, by letting the fluid run very slowly along the wall of the test tube. To each tube of the second row 0.1 cc of fresh normal rabbit serum is added in the same way. This control shows at once whether the precipitation obtained is a specific

one or whether it is due to other causes, principally the acidity of the extract. Control No. 11 of the first and second rows will show that the extract by itself does not give rise to precipitation and control No. 12 of the first row will show that normal rabbit serum plus NaCl is also absolutely free of any precipitate. With a large number of controls, the security of the results is warranted. The test tubes are now put into an incubator at 37°C. Before the reading of the reaction, in order to avoid autosuggestion on the part of the examiner, the identification marks on the test tubes are carefully concealed. A reading is taken every hour for the first 6 hours and then after 12 hours. As a rule, when the immune serum has a high titer, soon after the addition of the immune serum, a slight turbidity is noticeable. This gradually increases and, after a few minutes, gives rise to fine precipitates which are very manifest already after 15 minutes. Naturally, if the reaction is a negative one, precipitins are not formed and the fluid in the test tubes remains absolutely clear even after 24 hours. At times, slight turbidities may be noticed, which are to be interpreted as negative or false reactions, since a definite precipitin reaction is characterized by the presence of fine, minute, whitish particles, like floccules, suspended in an absolutely clear fluid. These can be seen best through a magnifying lens by holding the test tube against a black ground and comparing it with the control tubes.

Conglutination reaction

The school of MEZ has adopted a modification to the precipitin reaction, which is called "conglutination." If to the dilution of precipitinogen and its immune serum, as used with the precipitin test, a small and constant amount of ox serum be added, owing to the property of the "conglutinins" contained in the fresh active ox serum, the precipitation of the extract with its immune serum is more evident and the time of the reading of this reaction is shortened from 24 to 2.5 hours. The following is the technique used by them: One cubic centimeter of the extract of a uniform concentration is put into each of 5 small test tubes. To the first tube is added 0.08 cc of the immune serum; to the second, 0.02 cc; to the third, 0.01 cc; and to the fourth, 0.005 cc. The tubes are then incubated for 2 hours at 37°C and after this incubation each tube receives 0.4 cc of fresh ox serum. The tubes are again placed in the incubator and a reading taken every 20 minutes for 150 minutes.

Although MEZ and GOHLKE admit that the conglutination is "more complicated than the precipitation," yet they do not think that the addition of ox serum in the conglutination reaction has ever been the cause of

any disturbance in the results. In fact, they have carried out all the serodiagnostic researches in plants with the conglutination method as the principal reaction, using the precipitin reaction as a control to the first.

As the precipitin phenomenon represents a specific reaction by itself, I endeavored to adhere to the standardized rules of technique of this reaction. The principle adopted in my research, of working with antigen containing large and determined amounts of albumin, has always given me very active immune sera. Although I adopted the precipitin reaction as the main means of investigation in my research, on several occasions I tried the conglutination method as advocated by the school of MEZ. In fact, I used the sera of both oxen and sheep and, although I must avow that a certain improvement in the reaction is noticeable in the way of reading, yet, I do not agree, after all, that the slight improvement in the reaction can justify the introduction of another factor, like an extra animal serum with all its unknown sources of possible errors, which might leave the validity of the results obtained open to criticism.

RUEHLE (1924), in a publication regarding the relationship of the members of the subfamily Prunoideae, made an anatomical study of the seeds, concluding that the development in the position of the seeds (Samenlagen) of the *Prunus domestica* and the *Prunus armeniaca* is the same and that the relationship between the Pomaceae and the Prunoideae is confirmed by the presence of the obturator, the nucellar and the chalazal haustoria.

Although in my researches, with the precipitin reaction, it was easy to obtain a positive result between the various members of the Prunus subfamily, I did not succeed in obtaining a similar result between the Prunoideae and the Pomaceae. The absence of a precipitin reaction between these two subfamilies seems to coincide with the difficulty encountered in the intergrafting of these two subdivisions of the same family. The explanation of this mysterious phenomenon among plants, which, by their anatomical and other morphological characteristics would appear to have had a common origin and development, but whose common origin is not confirmed by immunological reaction, is probably to be sought in the occurrence of stereoisomeric forms of proteins and carbohydrates, as REICHERT (1916) propounds in the following quotation:

". . . . each kind of substance may exist in a number of forms, all of which forms have the same molecular formula and the same fundamental properties in common, but each in accordance with variations in intramolecular configuration has certain individualities which distinguish it from others. There are many known substances that exist in stereoisomeric forms and it has been found that the number of possible forms of each substance is dependent upon

the possible number of variations of the arrangements of the molecular components in the three dimensions of space, or, in other words, of variations of molecular configuration, the possible number in case of each substance being capable of mathematical determination. Thus, we find that serum-albumin may exist in as many as a thousand million forms. Haemoglobin, the red coloring matter of vertebrate blood, is a far more complex carbon compound than serum-albumin, and theoretically may exist in forms whose number is beyond human conception, running into millions of millions. The same is true of starch."

In order to investigate more deeply this very important subject, a large series of grafting experiments ought to be carried out with plants giving the same precipitin reaction, and especially to try to solve the problem why it is more feasible to graft the quince than the apple on the pear, researches which unfortunately I did not have an opportunity of pursuing. With a methodical serological aid I think a more definite knowledge of this curious phenomenon could be obtained. As ZIEGENSPECK (1925) says:

"The serological system throws light over much which up to the present time was problematic and gives us good prospects for further investigation into the history of our vegetation."

RESULTS AND CONCLUSIONS

The results of my studies are tabulated in table 1 from which the following conclusions seem to be justified:

(1) Plants of the genus Citrus, known to be easily intergrafted, gave a uniform positive precipitin reaction with an immune serum obtained from one of them.

(2) The subfamily of the Prunoideae, also known to intergraft successfully among themselves, although giving a uniform positive precipitin reaction among members of the same subfamily, gave a negative result when made to react with members of the subfamily Pomoideae. The Pomoideae seem to be an independent entity from the Prunoideae.

(3) The specimens of the Solanaceae examined, also known to intergraft, gave also a positive precipitin reaction.

(4) The control specimens of *Beta vulgaris* (beet), of *Rheum officinale* (rhubarb) and of *Olea europaea* (olive), belonging to three different families, gave always a negative result with each of the immune sera of the other families investigated in this research.

TABLE 1

Precipitin reaction.

PRECIPITINOGEN OF THE	IMMUNE SERUM AGAINST *C. decumana* TITER 1 : 12,800	IMMUNE SERUM AGAINST *P. amygdalus* TITER 1 : 12,800	IMMUNE SERUM AGAINST *Pyrus malus* TITER 1 : 6400	IMMUNE SERUM AGAINST *Lycopersicum esculentum* TITER 1 : 6400	NORMAL RABBIT SERUM
Family: Rutaceae					
Genus: Citrus					
C. decumana	+++	−	−	−	−
C. aurantium	+++	−	−	−	−
C. limonum	+++	−	−	−	−
C. vulgaris	+++	−	−	−	−
C. nobilis	+++	−	−	−	−
Family: Rosaceae					
Subfamily: Prunoideae					
P. amygdalus	−	+++	−	−	−
P. armeniaca	−	+++	−	−	−
P. persica	−	+++	−	−	−
P. domestica	−	+++	−	−	−
Family: Rosaceae					
Subfamily: Pomoideae					
Pyrus malus	−	−	+++	−	−
Pyrus communis	−	−	+++	−	−
Family: Solanaceae					
Lycopersicum esculentum	−	−	−	+++	−
Solanum tuberosum	−	−	−	+++	−
Family: Chenopodiaceae					
Beta vulgaris	−	−	−	−	−
Family: Polygonaceae					
Rheum officinale	−	−	−	−	−
Family: Oleaceae					
Olea europaea	−	−	−	−	−

Note: +++ indicates a positive reaction.
− indicates a negative reaction.

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GAMETOPHYTIC INHERITANCE IN SPHAEROCARPOS. III THE GENETIC UNIFORMITY OF TYPICAL CLONES

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INTRODUCTION

Among the f_1 (gametophytic) progeny of a mating between female and male clones selected as typical for *Sphaerocarpos Donnellii*, a few clones appeared which have hitherto been classed as "possibly atypical." As previously noted (ALLEN 1924, p. 559), such f_1 clones were observed to differ from those classed as "typical" only by displaying at some time or times in their history an unusually large proportion of variant involucres. Similarly doubtful clones of one or of both sexes have appeared among the offspring of matings of tufted ♀ × typical ♂ (ALLEN 1924), typical ♀ × polycladous ♂, and tufted ♀ × polycladous ♂ (ALLEN 1925). The possibility was suggested (ALLEN 1924, p. 561) that the differences between "typical" and "possibly atypical" races might be expressive of new combinations of factors with respect to which the "typical" ancestors differed.

If the phenotypic differences in question have a genetic basis, it would be expected that a mating between one of the doubtful clones and a typical clone of opposite sex would give results different from those of a mating between two typical clones. The experiments here reported were planned to test this possibility.

Improved methods developed within the past three years have, among other advantages, made it possible to secure the germination of a larger proportion of the spores sown. The improvement consists chiefly in maintaining a constant flow of water over the bottoms of enameled pans, each pan fitting closely one of the Wardian cases in which are set the pots of

soil containing the spores, spore tetrads, or growing plants. The pots thus stand in a constantly renewed supply of water from one-fourth inch to one inch in depth. In this way the temperatures of the soil as well as of the atmosphere within the case are kept lower than when the pots are set in standing water. The lower temperatures seem to be un-

TABLE 1

Gametophytic offspring of matings 16, 17 and 18.

OFFSPRING (f_1)	TETRADS SOWN SEPARATELY (20 FROM EACH CAPSULE)				TOTALS (TETRADS)
	MATING 16	MATING 17		MATING 18	
	CAPSULE A	CAPSULE A	CAPSULE B	CAPSULE A	
2♀, 2♂	2	3	2	..	7
2♀, 1♂	1	4	1	..	6
2♀	6	3	1	..	10
1♀, 2♂	1	1	3	..	5
1♀, 1♂	1	1	3	1	6
1♀	5	2	3	..	10
2♂	1	3	..	..	4
1♂	2	2	3	..	7
Totals	19	19	16	1	55

Gametophytes possibly atypical: Mating 16, 1♀; mating 17, capsule A, 3♀; capsule B, 2♀. Character undetermined (died young): Mating 17, capsule A, 1♂. All others (61♀'s, 50♂'s) typical.

	TETRADS SOWN BROADCAST				TOTALS (GAMETOPHYTES)	
	MATING 16	MATING 17		MATING 18		
	CAPSULE B	CAPSULE B	CAPSULE C	CAPSULE B		
♀ typical	30	38	63	22	153	
♀ possibly atypical	3	2	2	..	7	161
♀ character undetermined	..	..	1	..	1	
♂ typical	6	36	42	8	92	
♂ polycladous	..	..	..	1	1	98
♂ character undetermined	1	4	..	..	5	
Totals	40	80	108	31		259

All ♀'s (from sowings of both types): typical, 214; possibly atypical, 13; character undetermined, 1; total, 228.

All ♂'s: typical, 142; polycladous, 1; character undetermined, 6; total, 149.

favorable to the development of the algae which have been the most troublesome source of contamination, while they do not noticeably hinder the growth of Sphaerocarpos,—as had been previously shown in experiments devised by Doctor H. W. RICKETT. At first the water used was that obtained by the university pumping station, from Lake Mendota; more recently the culture cases have been connected with the city water supply, which is derived from artesian wells and is practically sterile.

Some of the spore tetrads from each mating were sown separately, and the classification according to sex of the offspring of each tetrad is shown in the first part of table 1. It may be noted here that the results are in harmony with the expectation, based upon previous work, that of each tetrad two spores will develop into female, and two into male gametophytes. Other tetrads were sown broadcast; the classification of their offspring is shown in the second part of table 1.

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DOUBTFUL (POSSIBLY ATYPICAL) ♀ × TYPICAL ♂

Mating 16. 20·337 × 21·162

Fertilized March 31, 1922. Female plants with mature sporophytes dried June 26. Spore tetrads sown February 2, 1923. Sporelings transplanted to separate pots May 3 to May 19.

Both clones used in this mating were among the offspring of a cross of typical × polycladous (mating 13, ALLEN 1925). Clone 20·337 (the maternal clone in the present mating) showed at times a large proportion of irregularities in involucral form; the paternal clone was at all times classed as typical.

The results of this and of the succeeding matings are shown in table 1. Twenty tetrads from one capsule (A) were sown separately, one to a pot, and the sporelings, after germination, were transplanted separately. The tetrads of a second capsule (B) were scattered upon the soil in a single pot; when the sporelings appeared, they were isolated without reference to their possible tetrad relationships.

Mating 17. 21·153 × 21·190

Fertilized May 12, 1922. Female plants with mature sporophytes dried July 4. Spore tetrads sown November 3 and November 18. Sporelings isolated March 5 to April 10, 1923.

The maternal clone was an offspring of the same cross (mating 13) of typical×polycladous, that gave rise to both clones used in mating 16. This clone (21·153) was described in a previous paper (ALLEN 1925, p. 8) as one whose frequent aberrancies gave rise to doubt concerning its nature. It was finally classed as "possibly atypical."

The paternal clone (21·190) in the present mating was an offspring of typical×typical (mating 1, ALLEN 1924). Its typical character was at no time doubtful.

Twenty tetrads of capsule A and 20 of capsule B were sown separately. The remaining tetrads of capsule B and all those of capsule C were sown broadcast.

TYPICAL ♀ ×DOUBTFUL ♂

Mating 18. 20·275×20·340

Fertilized March 31, 1922. Female plants with mature sporophytes dried June 23. Tetrads sown February 1, 1923. Sporelings isolated May 15 and May 19.

Both clones used in this mating were offspring of mating 13 (typical ×polycladous). The female clone (20·275) appeared at all times substantially typical. The male clone was the subject of some doubt; at the time of one observation, in particular, it displayed an unusual proportion of aberrant structures, but was finally classed provisionally as typical.

Twenty tetrads from one capsule (A) were sown separately. All the tetrads from a second capsule (B) were sown broadcast.

DISCUSSION

The results of these matings show that "possibly atypical" clones mated with typical clones of opposite sex behave genetically substantially as do typical clones similarly mated. In either case, intraclonal variation among some of the gametophytic offspring is apparently greater than the variation within a single typical clone,—which fact gave rise to the suspicion that some of the apparently more variable clones represent new factorial combinations. This suspicion is not supported by the results here described. The differences noted between "typical" and "possibly atypical" clones seem to be purely phenotypic. The maternal clone (21·153) in mating 17 has displayed perhaps a greater degree of aberrancy than have any of the other doubtful clones of the "possibly atypical" class, yet the proportion among its progeny that can be suspected of being

other than typical is no greater than among the progeny of a typical clone similarly mated.

So far as present evidence goes, therefore, the great majority of the plants of *Sphaerocarpos Donnellii* must be considered to be genetically similar,—at least as the species is represented about Sanford and Miami, Florida, the localities from which my material was derived. The only genetically distinct form that occurs, other than very rarely, seems to be the tufted one (ALLEN 1924). Clones of the sort hitherto classed as "possibly atypical" will hereafter, therefore, be treated as typical.

That genetic differences may exist between some of these clones, as well as between other clones ranked as typical, cannot be denied. But such differences, if they exist, are not distinguishable by criteria now available. It is also true that, since strains exist which possess, or at least express, the tufted tendency in a very slight degree, some tufted clones may be classed, from their appearance, even over a considerable period, as typical. Hence, it is to be expected that in an occasional instance the classification of a clone as typical may be found to be erroneous.

The one male clone listed as polycladous among the progeny of mating 18 was apparently the result of a mutation. However, this clone was very small and unhealthy throughout its life, and it is possible, though not probable, that its determination was erroneous. The possibility of an accidental contamination is also not excluded.

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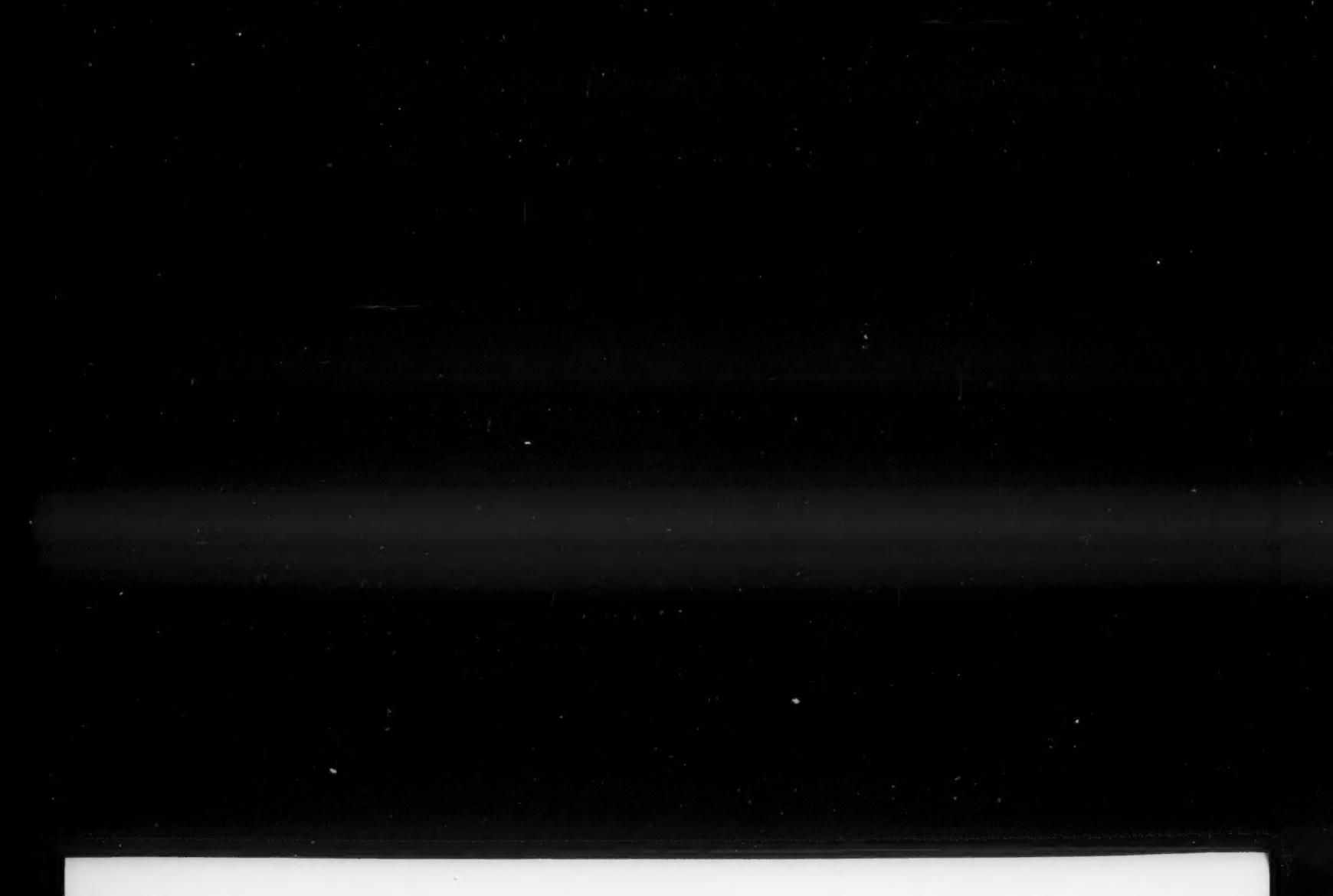
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